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VOLUME XXV NOVEMBER, 1938 No. 4

COMPARATIVE EFFECTS OF COBALT, NICKEL AND COPPER ON PLANT GROWTH

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(With Plates XXIII–XXV and 4 Text-figures)

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A. INTRODUCTION

A NOTEWORTHY development of modern physiology has been the recognition of the part played by elements, other than those generally accepted as nutritive, in the economy of the plant. The work has developed along two apparently contradictory lines, in connexion with the toxic effect and the beneficial action of the elements, as in some proved cases the same element may function either way according to circumstances. This contradiction is only apparent, since it is generally accepted that most, if not all, elements, nutritive or otherwise, are poisonous if present in excess, though the limit varies widely with different elements. Attention is now frequently concentrated on the "trace" or "minor" elements, which are often found in very small amounts in soils and plant tissues, and which in some cases are proving of great importance in plant development. Minute traces of boron and manganese are recognized as being essential for growth, and the possibility exists that other elements,

especially copper (Allison *et al.* 1927), may be equally necessary in certain cases. The quantities involved in this way are so small that their investigation needs special technique on account of the difficulty in eliminating unwanted traces of elements from the nutrient substratum. Occasionally, as happened with boron, a fortunate chance gives a "pointer" in the direction of profitable investigation but, more usually, it is necessary to narrow the investigation down from the toxic aspect to the possible beneficial function.

The available information as to the comparative effect of trace elements on plant life is scanty, except in a few cases. While very small amounts may be essential, or may benefit growth, larger quantities may exercise a toxic action which is often obscure, and difficult to recognize if the result is a depression in growth without visible signs of poisoning. The present investigation concerns the second of these possibilities. Cobalt and nickel are of general occurrence in soils, and have been credited with stimulating crops (Nakamura, 1904–5), while recently cobalt has attracted attention on account of its relation to certain animal diseases on soils in which it is deficient. Copper has been included in the work to provide a basis of comparison with an element of considerable economic importance on which more information is available.

B. Historical

The soil content of cobalt and nickel is extremely variable. Kidson (1937) reports a range of 0·3–380 p.p.m. of cobalt in New Zealand soil, the latter figure being outstandingly high. Bertrand & Mokragnatz (1922*a*) found 2·8 mg. of cobalt and 13·6 mg./kg. of nickel in a very fertile arable soil from Pantchevo, near Belgrade, and later (1925*a*) recorded a range of some tenths mg. to 11·7 mg. for cobalt and 5–38·6 mg. for nickel in arable soils from various parts of Europe. Cobalt always seems to be the less in quantity, the Co/Ni ratio varying in their tests from 1/2 to 1/8. High soil content of nickel, and possibly cobalt, may cause damage to crops, as Robinson *et al.* (1935) found that infertile soils derived from serpentine had physical properties that were favourable to plant growth, but contained large quantities of nickel and chromium, and, in one case, of cobalt. Hosking (1936) also determined the cobalt, nickel and copper content of various soils, giving details for both calcareous and non-calcareous types.

The recognition of cobalt as a plant constituent dates back nearly a century, when Legrip (1841) found it in *Lathyrus odoratus*. Forchhammer (1855) then discovered cobalt and probably nickel in the ash of oakwood,

and also stated that the nickel present in peat was very probably derived from the breaking down of vegetation. After another fifty years the more general distribution of these elements in the plant kingdom began to be recognized. Vernadsky (1922) found both nickel and cobalt in all the mosses and several of the phanerogams round Kief. McHargue (1925, 1927) made quantitative estimations, showing traces of the elements in *Poa pratensis* and the leaves of soybean; only a trace of cobalt occurred in soybean seeds, but 3·92 p.p.m. of nickel were present. He also made the suggestion that organic colloidal complexes of these elements, amongst others, might exercise important catalytic functions in the metabolic processes of plants and animals. The same hypothesis was put forward by Bertrand & Mokragnatz (1922*b*, 1925*b*, 1930) who determined the presence of cobalt and nickel in many plants, and concluded that these elements are universally distributed among both phanerogams and cryptogams. Both tend to accumulate in the leaves and seeds, but the quantities are very small, being usually about 1 part of nickel to several million parts of the living plant, while the amount of cobalt is from five to ten times less. Similar results as to the relative distribution of nickel and cobalt are quoted by Ramage (1936), who found nickel usually in traces in various spices, herbal drugs and tea, cobalt being present less frequently. Exceptional amounts occur in St Ignatius' beans, with 0·014% of nickel and 0·003% of cobalt. Martini (1930), again, found nickel to be a normal constituent of plant tissues, occurring in species of the most varied nature, and he also suggested the possibility that it is essential to plant life. Bishop & Lawrenz (1932), on the other hand, claim that while they usually found cobalt in New Zealand spinach, chard, Chinese cabbage and turnips, nickel was always absent, a somewhat surprising result in view of the general consensus of contrary opinion.

The literature on the effect of cobalt and nickel on plant growth is not very comprehensive, and largely consists of isolated observations in connexion with tests of other elements. According to Ducloux & Cobanera (1911–12) very dilute solutions of cobalt salts slightly stimulated *Pisum sativum*, but such stimulation was confined almost exclusively to the leaves, while the roots were usually depressed. In some cases cobalt was eventually found in the leaves as a storage product. Some stimulation of flax was observed by Fukutome (1904) when 0·02 g. of cobalt nitrate was added to 8 kg. of soil. Albano (1915) stated that small dressings of nickel improved the growth of *Corchorus capsularis* (jute), but the quantity recommended as beneficial works out at over 1 cwt. per

acre, which would appear to be a very considerable application. Loew (1924), on the contrary, found no stimulation of cereals with 0·01 g. of nickel sulphate per 2·3 kg. of soil, whereas ten times the quantity caused damage, both these figures being much lower than those of Albano.

All these suggestions of stimulation are somewhat vague, but the evidence for toxicity is clearer, but very scanty. Seedlings of olive trees grown in soil were damaged by cobalt or nickel chlorides applied either in the form of solution added to the normal water supply, or as powder sprinkled on the surface of the soil and carried down gradually by watering. Petri (1910) found that the young shoots were checked by cobalt, and also that an accumulation of the toxic substances occurred, especially in the older leaves, in which the chlorophyll was partly destroyed and the laminae partly dried out by the treatment. The harmful effect of water containing cobalt had already been shown by Haselhoff (1895), from 1 to 2 mg./l. cobalt being sufficient to kill maize and beans grown in water cultures.

The same worker (1893) demonstrated the injurious effect of nickel oxide on horse beans and maize, his concentrations ranging from 2·5 to 50 mg./l. Even the smallest dose poisoned the plants, killing the leaves and seriously hindering development. In the light of more recent work, however, 2·5 mg./l. is in reality a fairly large dose, being equivalent to 2·5 p.p.m., a strength which causes trouble with many compounds. With barley, which was germinated and grown under sterile conditions in nutrient solutions, Wolff (1913) tested nickel sulphate against iron sulphate. Whereas 0·4 g./l. of iron sulphate was very favourable to growth, 0·2 g. of nickel sulphate killed the plants, the result being the same when the concentration was lowered to 0·02 g./l. Cotton (1930) working with buckwheat, indicated that nickel was more toxic than iodine. In the stronger concentrations the stems and leaves lost their turgidity and the young plants died within a fortnight, even 5·87 p.p.m. of nickel being fatal to most plants. The survivors were stunted, newly developed leaves being yellow and covered with small brown spots. Still lower concentrations caused spotting and chlorosis of leaves, as well as depressed growth, and in some cases chlorosis was shown in the presence of as little as 0·59 p.p.m. of nickel. No evidence of stimulation was obtained with still lower concentrations.

Another aspect of toxicity was investigated by Niethammer (1930), who soaked seeds for certain periods in various concentrations of nickel and cyanogen compounds previous to germination. He found that the effect of nickel salts on different species depends upon whether the

solutions can penetrate to the embryo. Wheat grains are penetrated, in spite of their semipermeable membrane, and poisoning occurs. Onions are much less susceptible to parallel strengths, as their seed coats offer a great resistance to penetration. Thiocyanates also penetrate wheat grains, but are less poisonous than nickel salts, and with some strengths stimulation occurs. With older wheat grains, whose vitality has become lessened, nickel salts apparently fail to poison, and may even stimulate.

Results obtained by various workers indicate that compounds of cobalt, nickel and copper are less toxic to the lower than to the higher plants, and more definite evidence of stimulation by low concentrations is available (Ono, 1900; Richards, 1897). Manoilow (1907) found that nickel salts were considerably less poisonous to micro-organisms than those of copper or other metals. On the other hand, Clark (1899) claimed that both nickel and cobalt were more toxic to filamentous fungi than either copper or zinc, though the toxic strengths were considerably above those damaging the higher plants. With *Aspergillus niger* the poisonous action of nickel and cobalt varies considerably, as Mokragnatz (1931) found that though the harmful action of cobalt begins at a strength at which nickel is innocuous, a dose which completely checks the formation of mycelia is lower for nickel than cobalt.

At the present time the importance of cobalt for animal nutrition in certain areas is being so much emphasized that a brief review of the position is given here. The presence of cobalt and nickel in plants and animals was mentioned in a historical account given by Chevalier & Cottereau (1849), but little notice was taken till Bertrand & Mâcheboeuf (1925*a*, 1925*b*, 1926*b*) published a series of analyses showing the distribution of the two elements in various animal organs, both usually being present together. The liver and pancreas of man and the higher animals are the richest organs, nickel being more abundant than cobalt, and marine molluscs are also very rich in nickel. These findings were corroborated by later workers (Dutoit & Zbinden, 1929, 1930; Fox & Ramage, 1931), and nickel was reported by Martini (1929) to be a normal constituent of the mineral salts in bones. Bertrand (and Mâcheboeuf) (1926, 1926*a*) suggested an association of the activity of insulin with the presence of its content of nickel and cobalt, hinting at the possibility of curing certain forms of diabetes by the introduction of small quantities of these elements into the general circulation. Very small traces of nickel and cobalt appeared to be beneficial to white mice (Bertrand & Nakamura, 1927), but in larger amounts both elements come high in the scale of toxicity, the metabolism of rats (Bertrand & Nakamura, 1927; Stare & Elvehjem,

1933; Waltner & Waltner, 1929) and guinea-pigs (Bertrand & Serbescu, 1931) being severely affected, often resulting in death.

During the last few years special attention has been drawn to cobalt in relation to certain deficiency diseases in Australia and New Zealand. Morton Mains disease (Dixon, 1936, 1937*a*; Wunsh, 1937), coast sickness (Lines, 1935), bush sickness (Grimmett & Shorland, 1935), and enzootic marasmus (Underwood & Filmer, 1935) are all forms of sheep disease which have been treated successfully by the administration of small amounts of cobalt, either in the food or by means of drenches. Various investigators, especially Askew *et al.*, have shown, by analyses of soils and pastures, that the incidence of these diseases is associated with local cobalt deficiency, and active experimental work is still in progress (Askew & Dixon, 1936, 1937*a*, 1937*b*, 1937*c*; Askew & Maunsell, 1937; Bell, 1937). Feeding on pastures top-dressed with cobalt salts, drenching the animals with solutions of various types containing small amounts of cobalt, and the provision of cobaltized salt licks, have all proved to be effective methods of combating these diseases. Dixon (1937*b*) has also shown that small quantities of nickel added to a cobalt drench gives better results than cobalt alone. In this country Patterson (1937) states that sheep disease with similar symptoms occurs at Dartmoor on moorland soils with a cobalt content of 3·9 p.p.m., whereas the soils supporting healthy sheep have 16·7 p.p.m.

C. Experimental work

The object of the experiments was to determine the toxicity of nickel and cobalt in view of their widespread distribution in soil. Parallel tests were made with copper to provide a comparison with an element whose action on plant growth is relatively well known.

In considering the effect of various elements on plant growth one of the chief difficulties is the great variation in the nomenclature of the various solutions used, which renders comparison impossible without some form of reduction to a common basis. Every such basis can be criticized for some reason, but perhaps the one least open to objection is that in which the solution of standard strength contains the atomic weight of the element concerned in mg./l. This renders it possible to make direct comparisons between equivalent amounts of the element, regardless of the chemical combination in which it is presented. For the sake of convenience the symbol E has been adopted throughout this work for such a standard solution, so that E = a solution containing the atomic weight of any particular element in mg./l.

With compounds containing one atom of the test element per molecule E therefore represents an $M/1000$ solution, but in cases where two or more atoms are present the equivalent is $M/1000$ divided by the number of such atoms.

Tests were carried out in water cultures using a range of concentrations from $E/4096$ to E, each concentration being two or four times that of the preceding one. The usual Rothamsted nutrient solution was used, with pH 5 for barley and pH 6·2 for broad beans, and the experiments extended over two growing seasons, arranged as follows:

		Barley	Broad beans
1935			
Nickel sulphate, Cobalt chloride, Copper sulphate	In solution	6 March	16 July
	Harvested	18 and 30 July	12 October
1936			
Nickel chloride, Cobalt sulphate, Cupric chloride	In solution	5 March	23 July
	Harvested	24–27 July	23 October

The growing periods for each crop in the two years were very similar in length. In 1935 most of the barley was cut on 18 July, but some plants that were still very immature at that time were left until 30 July before harvesting. In 1936 the whole crop was cut at once, since the previous year's experience had shown that little was gained by delay.

Detailed description of the individual experiments is unnecessary. The control plants and those with concentrations too low to be toxic grew normally, producing good ripe ears in barley, and strong healthy growth in broad beans. The response to toxicity, however, varied with the element and with the species, and some variation also occurred between the compounds in which the elements were presented.

(*a*) *Barley*

The general trend of response to the action of sulphate and chloride showed certain similarities for each of the three elements, but varied slightly between the compounds (Table I).

(1) *Sulphates.*

With the sulphates, growth was usually much the same in the control solutions and some of the weakest concentrations, after which a marked toxic action became manifest, and the growth curves dropped very suddenly to a point where very little development occurred before the plant died (Text-fig. 1). With nickel sulphate there was a very slight significant fall in the total dry weight between $E/1024$ and $E/64$, the depression being confined to the shoot, not occurring in either root or

Table I. *Dry weights of barley. Grown February–July* 1935, 1936

(Means of 5 plants, in g.)

Concentration	Shoot	Root	Grain	Total	Shoot	Root	Grain	Total
	Cobalt							
	Sulphate (1936)				Chloride (1935)			
Control	26·80	4·50	15·83	47·13	25·13	4·28	14·73	44·14
E/4096	27·05	4·13	16·61	47·79	25·75	4·28	15·42	45·45
E/1024	25·54	4·11	15·39	45·04	23·93	3·50	13·77	41·20
E/256	27·27	4·87	16·77	48·91	24·15	4·18	14·71	43·04
E/64	28·37	4·31	15·60	48·68	20·62	3·99	13·04	37·65
E/32	20·03	2·23	5·60	27·86	*			
E/16	0·31	0·09	—	0·40	7·01	1·02	1·13	9·16
E/8	0·12	0·02	—	0·14				
E/4	0·06	0·01	—	0·07	0·09	0·02	—	0·11
E					—	—	—	—
	Nickel							
	Sulphate (1935)				Chloride (1936)			
Control	23·30	3·37	14·32	40·99	26·55	4·31	16·76	47·62
E/4096	23·84	3·73	13·23	40·80				
E/1024	23·92	3·85	14·15	41·92	27·22	4·16	17·10	48·48
E/256	21·19	3·36	12·92	37·47	25·48	3·81	15·88	45·17
E/64	20·18	3·36	13·23	36·77	23·36	3·29	14·25	40·90
E/32					22·59	2·60	11·95	37·14
E/16	9·80	1·70	3·53	15·03	8·99	1·13	0·77	10·89
E/8					0·07	0·02	—	0·09
E/4	0·04	0·01	—	0·05	0·05	0·01	—	0·06
E	—	—	—	—				
	Copper							
	Sulphate (1935)				Chloride (1936)			
Control	21·73	3·93	13·44	39·10	25·31	4·39	16·40	46·10
E/4096	22·24	3·66	13·19	39·09				
E/1024	21·40	3·41	12·29	37·10	24·04	4·12	15·17	43·33
E/256	22·14	3·88	13·44	39·46	21·73	3·00	12·45	37·18
E/64	16·67	2·68	8·29	27·64	21·71	2·93	9·22	33·86
E/32					10·08	1·44	2·15	13·68
E/16	0·08	0·03	—	0·11	0·75	0·28	—	1·03
E/8					0·14	0·05	—	0·19
E/4	—	—	—	—	0·09	0·03	—	0·12
E	—	—	—	—				

* A gap in the table indicates that no experiment was carried out at that concentration, whereas a dash shows that plants were put in nutrient solution but failed to develop. This applies to all tables.

grain. With nickel and cobalt the abrupt decrease in weight set in above the *E*/64 limit, but the toxicity of cobalt was the greater, and *E*/16 practically checked any development. At this strength the plants with nickel sulphate still gave an average dry weight of 15 g., but were very variable, the individuals ranging from 2·0 to 25·68 g. in weight, while at *E*/4 the seedlings died without developing at all. (Pl. XXIII, fig. 2.) Copper sulphate proved to be more poisonous than either of the others,

as the abrupt fall set in between $E/256$ and $E/64$, and practically no growth occurred at $E/16$ (Pl. XXIII, fig. 3).

The greater toxicity of copper showed at an early stage, before the plants had been in the solutions for a week. Checking of root growth was manifest at low concentrations which allowed perfect growth with nickel or cobalt, and the trouble became accentuated as time went on. With nickel sulphate white lesions appeared on the leaves at an early date with $E/16$ and stronger solutions, the roots also being checked, and shortening

Text-fig. 1. Total dry weights of barley grown with varying concentrations of sulphates. —•—•— cobalt; - - - - - nickel; ——— copper.

Text-fig. 2. Total dry weights of barley grown with varying concentrations of chlorides. —•—•— cobalt; - - - - - nickel; ——— copper.

of roots appeared later with $E/64$, but did not become very marked. With cobalt sulphate a tendency to checked root growth was soon shown in solutions as dilute as $E/64$ or even $E/256$. The leaves were not injured and eventually the roots grew normally. In higher concentrations the toxic effect was again first shown in the roots, the leaves becoming affected somewhat later.

The toxic action of the three elements was more marked in the grain than in either the shoot or root. With the weakest strengths no harm was done, but with the first sudden drop in dry weight the grain was so depressed as

to lower considerably the proportion between the dry weights of the grain and the whole plant (Table II). With the higher concentrations tested no grain at all was produced in association with the very small shoots and roots.

Table II. *Barley.* % *of grain in whole plant*

Concentration	Sulphate			Chloride		
	Cobalt	Nickel	Copper	Cobalt	Nickel	Copper
Control	31·89	34·94	34·37	33·37	35·20	35·56
E/4096	34·76	32·42	33·75	33·93		
E/1024	33·80	33·76	33·13	33·42	35·28	36·25
E/256	34·29	34·49	34·05	34·17	35·16	33·49
E/64	32·86	35·98	29·98	36·63	34·85	27·25
E/32	20·10				32·17	15·75
E/16	—	23·50	—	12·30	7·09	—
E/8	—				—	—
E/4	—	—			—	—
E		—	—	—		

With the concentrations of sulphates that caused marked depression toxic symptoms appeared from the outset, shortened roots being well marked within five days, often accompanied by lesions on the leaves or abnormal, stiff shoot development. In every case this damage persisted and became intensified as time went on. With the borderline concentrations in which the plants were ultimately as good as the controls, as judged by the dry weights, temporary symptoms of toxicity occurred at some stage. With nickel and copper slight symptoms appeared about a fortnight from the start, lasting only a few days with nickel, but persisting for 8 weeks with copper, finally disappearing without doing any permanent damage. With the parallel strength of cobalt 4 weeks elapsed without any determinate signs of toxicity, and then the plant became slightly chlorotic for about a fortnight, soon recovering and producing normal plants.

(2) *Chlorides.*

The range of low concentrations which were innocuous in the case of the sulphates exhibited a certain degree of toxicity when the chlorides were used (Table I). With copper chloride there was a steady significant fall from the control to *E*/64, with nickel chloride from *E*/1024 to *E*/32, whereas, with cobalt chloride, the slight drop was only significant from *E*/256 to *E*/64. (Text-fig. 2). Beyond this range the sudden depression occurred, just as with the sulphates, very little or no growth being made in the highest concentrations.

The visible signs of toxicity were slow in appearing with cobalt chloride, as lesions on the leaves and checking of root growth were not

exhibited so early as with the other elements. Later on, concentrations which at first appeared harmless began to act, and the characteristic marks of poisoning appeared. With concentrations at which marked toxicity first appeared great variation occurred between the individuals with the same treatment, that with the chloride of cobalt and copper being more striking than in the case of the sulphates (Table III, Pl. XXIII, figs. 1, 3).

Table III. *Range of variation between the dry weights of individuals at critical concentrations, in g.*

	Sulphate		Chloride	
	Concentration	Range	Concentration	Range
Cobalt	*E*/32	20·2–30·9	*E*/16	4·3–14·7
Nickel	*E*/16	2·1–25·6	*E*/16	1·6–17·7
Copper	*E*/64	15·2–36·4	*E*/32	6·1–23·7

The critical point beyond which the major depression occurred was much the same with both compounds, but varied with the elements. With cobalt it was *E*/64, and with nickel *E*/64 for sulphate, and *E*/32 for chloride. No test was made with *E*/32 nickel sulphate, and it is possible that this might have been the true critical point for this compound. Copper was the only one in which a marked dissimilarity in behaviour occurred, as the strong depression set in beyond *E*/256 with the sulphate, and *E*/64 with the chloride. Even here, however, the discrepancy was not very marked, owing to the considerable preliminary fall in dry weight with the lower concentrations of copper chloride.

The different effect of the various elements in strong concentrations is well seen by comparing Pl. XXIV, figs. 1–4, all taken on the same day when the plants were 15 weeks old, and the control plants were finely developed and had already produced ears. Even with *E*/4 the different action was manifest, the short bunchy root development with copper being specially marked. This "bunchiness" is caused by the abrupt checking in the growth of the first formed roots, succeeded by the production of a number of subsidiary roots which, in their turn, are checked at about the same stage of development. If the toxic agent is sufficiently strong this bunchy root may persist throughout the life of the plant. With somewhat weaker concentrations, however, the plant eventually becomes strong enough to develop more freely, and the later formed roots begin to elongate in the nutrient solution. In some cases the ultimate root produced may be almost normal in type, showing little signs of the preliminary bunching of the roots. This type of growth is characteristic with many poisonous substances, including copper.

Nickel and cobalt do not come into this category, as even with the strongest concentration the roots were very thin and elongated, the toxic action being manifested in the general feebleness of growth. With decreasing concentration the nickel plants improved rapidly, till at $E/32$ they were too big for comparison on the same photographic plate as the others. The general development with cobalt was similar, but the improvement was less rapid, and at $E/32$ growth was still below normal, indicating the greater toxicity of cobalt compared with nickel. The plants with copper retained their characteristic bunchy roots and depressed growth to $E/32$ and beyond, the improvement in the shoot setting in first.

Nickel and copper chlorides behaved like the sulphates, in that definite toxic symptoms appeared at the outset in the lowest concentrations which ultimately caused great depression, the trouble becoming accentuated with time. With cobalt, toxicity at the early stage was much less definite, 2 or 3 weeks elapsing before it became marked, but the ultimate poisoning was as severe as with nickel. With some of the weaker concentrations of all three chlorides toxic symptoms set in from two to four weeks from the beginning of growth, persisting but not becoming acute, and resulting in the gradual depression of dry weight already discussed.

Comparison of the growth curves shows that, under the conditions of the experiment, the order of decreasing toxicity of the three elements is copper, cobalt and nickel, with both the compounds tested (Text-figs. 1, 2). The extra toxicity of copper persists to a weaker concentration than with either nickel or cobalt. On the other hand, the greater toxicity of cobalt than nickel sulphate is shown most clearly in the higher concentrations, as $E/16$. The true effect of the higher concentration of cobalt chloride is uncertain, as $E/8$ was unfortunately omitted from the test, but judging by the usual slope of the curves it seems probable that very little growth would have occurred at this concentration. The extra toxicity of cobalt is again indicated by the marked depression in growth setting in at a weaker concentration than with nickel chloride. The differences in the weights of the various controls are due partly to the better growth in 1936 than in 1935, and partly to positional effect within the glasshouse. Each individual test, however, was concentrated and randomized, so that comparisons of the effects of treatment are valid.

(*b*) *Broad beans* (Vicia faba)

The tests with broad beans resembled those with barley, except that the weakest concentrations of the toxic substances were omitted, being replaced by more intermediate strengths. Well grown, healthy broad beans always exhibit considerable variations in size, so that no account can be taken of the fluctuations in the dry weights between the control plants and those in the weaker concentrations. The points of sudden

Text-fig. 3. Total dry weights of broad beans grown with varying concentrations of sulphates. —•—•— cobalt; - - - - - nickel; ——— copper.

depression, on the other hand, were definitely marked and the course of events with the higher concentrations is clear.

With barley, when once the borderline of toxicity had been passed, the growth curve fell steeply and almost uniformly till the lethal point was reached. With broad beans, the steep fall in the curve again occurred, but as the concentration increased the toxic effect did not increase to correspond, so that more growth was made at the higher strengths than was the case in barley (cf. Text-figs. 1, 3; 2, 4). As will be seen later, this

indicated a greater resistance of broad beans than barley to heavy doses of the poisonous salts tested.

(1) *Sulphates.*

Broad beans varied considerably in the rapidity of their exhibition of damage from poisoning. With nickel sulphate, in less than a fortnight the plants with $E/16$ were definitely poisoned, the roots being stunted and the leaves patchy in colour. As time went on, great depression in

Text-fig. 4. Total dry weights of broad beans grown with varying concentrations of chlorides. —•—•— cobalt; - - - - - nickel; ——— copper.

growth was accompanied by a narrowing of the newly formed leaves which were pale and mottled, the roots being short, dark and stubby. By harvest time these effects were intensified, though some plants had produced a few elongated laterals, usually of a thickened type. Weaker concentrations did not at first have any effect, but after about 6 weeks' growth the plants in $E/32$ and $E/64$ showed some thickening and shortening of roots, together with a weakening and paling of the shoots. These effects were temporary, however, as when harvested the plants were

Table IV. *Dry weights of broad bean* (Vicia faba). *Grown July–October* 1935, 1936

(Means of 5 plants, in g.)

Concentration	Shoot	Root	Total	Shoot	Root	Total
	Cobalt					
	Sulphate (1936)			Chloride (1935)		
Control	11·76	3·95	15·71	13·74	4·40	18·14
E/512	12·42	3·70	16·12			
E/256	11·27	2·89	14·16	13·57	4·32	17·89
E/128	3·57	0·81	4·38			
E/64	2·08	0·42	2·50	3·74	1·01	4·75
E/32	1·53	0·39	1·92	1·91	0·58	2·49
E/16	1·06	0·19	1·25	1·86	0·52	2·38
E/8	0·88	0·18	1·06	1·18	0·23	1·41
E/4	Not separated		0·65	1·08	0·15	1·23
E/2	,,	,,	0·41	—	—	—
E						
	Nickel					
	Sulphate (1935)			Chloride (1936)		
Control	16·20	4·11	20·31	14·14	4·16	18·30
E/512				15·60	5·00	20·60
E/256	18·40	5·26	23·66	11·93	3·69	15·62
E/128				16·48	4·59	21·07
E/64	14·40	5·10	19·50	15·68	5·16	20·84
E/32	13·47	4·83	18·30	5·25	2·06	7·31
E/16	4·23	1·07	5·30	2·16	0·88	3·04
E/8	2·42	0·68	3·10	1·23	0·52	1·75
E/4	1·00	0·19	1·19	Not separated		0·90
E/2	—	—	—	0·32	0·07	0·39
E						
	Copper					
	Sulphate (1935)			Chloride (1936)		
Control	18·32	5·06	23·38	13·99	4·82	18·47
E/512				17·01	5·49	22·50
E/256	15·07	4·52	19·59	14·67	4·46	19·13
E/128				14·39	3·55	17·94
E/64	15·71	5·04	20·75	15·27	4·10	19·37
E/32	8·37	2·52	10·89	14·27	3·18	17·45
E/16	5·06	1·22	6·28	7·60	2·07	9·67
E/8	5·13	1·45	6·58	3·86	1·05	4·91
E/4	3·83	1·16	4·99	2·66	0·96	3·62
E/2	1·25	0·43	1·68	1·59	0·65	2·23
E	—	—	—	0·50	0·34	0·84

healthy and normal, except that those with *E*/32, just below the borderline of toxicity, were somewhat unusually variable. After the abrupt drop in weight at *E*/16, the toxic effect decreased, and total inhibition of growth did not occur till *E*/2 (Table IV, Text-fig. 3). Copper sulphate, near the critical strength, was much slower in coming into action. With *E*/32, which finally caused marked depression in growth, no ill-effects were obvious for a long time, since after 6 weeks' growth the roots were

Table V. *Heights of broad bean* (Vicia faba)

Mean of 5 plants, in cm.

	Cobalt	Nickel Sulphate	Copper
Control	82·9	80·8	97·3
E/512	73·1		
E/256	79·6	81·4	82·6
E/128	49·4		
E/64	38·4	81·6	92·7
E/32	28·1	79·3	76·3
E/16	24·9	41·3	61·7
E/8	19·5	32·4	61·0
E/4	11·9	15·0	54·3
E/2	7·0	—	33·0
E	—	—	—
		Chloride	
Control	80·1	102·3	104·1
E/512		101·4	113·7
E/256	74·0	90·0	100·1
E/128		91·4	106·0
E/64	50·5	89·5	99·3
E/32	33·8	42·1	112·5
E/16	37·4	31·9	91·4
E/8	17·5	25·9	69·6
E/4	18·8	8·2	57·5
E/2	—	3·5	46·3
E	—	—	4·5

only beginning to become short and bunchy, and the shoot to produce narrow and pale leaves. By harvest time the growth was considerably less reduced than is usual when marked toxicity sets in. After a continued rapid drop to *E*/16, the depression of growth was slower, and the plants were much larger than with similar strengths of nickel sulphate, *E* being the only concentration which killed the plants outright at the beginning. From *E*/8 to *E*/2 the roots were typical, with short, thick laterals, often curved, bunched together on the main root. The tips of the laterals were often curved, the apices often swollen, while the cortex of the main root was frequently split from the pressure of the mass of rootlets trying to emerge from the stunted root. All these malformed roots were dark brown or black in colour, and were accompanied by tall shoots which showed a marked contrast in size with the corresponding shoots in nickel and cobalt sulphate (Table V).

The poisonous effect of cobalt sulphate was even slower in manifesting itself than that of copper sulphate. Five weeks elapsed before the plants with *E*/128 showed any sign of trouble, and then they were rather more variable than normally and the roots were beginning to thicken. A fortnight later they were still as tall as the controls, but were turning yellow

and some of the apical leaves were withering. Yet, when harvested in October, great depression of growth had occurred, and most of the shoots were much shorter than the controls and were dead or dying, indicating severe poisoning (Pl. XXV, fig. 1). This behaviour affords a good instance of the danger of drawing conclusions with regard to toxicity or stimulation from plants that are too young and immature. The absence of symptoms at an early stage does not necessarily imply inaction, as trouble or benefit may set in at a later stage of growth. In this case, the effect of nickel sulphate was manifest at an early date and persisted throughout whereas, with copper and cobalt sulphate, concentrations that at first appeared innocuous proved later to be strongly toxic.

(2) *Chlorides.*

The gradual depressing action of low concentrations of the chlorides, which occurred with barley, was not evident with broad beans (Text-fig. 4). The dry weight with the strongest concentration that did not cause marked depression was in no case significantly below that of the control (Table IV). With nickel and cobalt the onset of the toxic symptoms was somewhat slow, three or four weeks elapsing before the plants showed the characteristic variability and definite signs of poisoning. At this time $E/32$ nickel chloride had caused much shortening and thickening of the roots, while the leaves were yellowish and mottled. With age the symptoms were intensified and numerous small brown spots appeared on the apical leaves. With stronger concentrations the thickening of the roots was abnormal, but some of the plants just managed to survive at $E/2$ (Pl. XXV, fig. 2).

Copper chloride proved innocuous to $E/32$, but toxic symptoms appeared almost at once with $E/16$ and persisted throughout growth (Pl. XXV, fig. 3). As with copper sulphate, however, the behaviour differed from that with nickel and cobalt, since the depression was less great at the first toxic concentration and continued at much the same rate to the next, in this case $E/8$, after which the associated fall in dry weight decreased considerably. Even at $E/2$, the plants had shoots 46 cm. long, but E had prevented any development of root or shoot. The strengths at which the elements allowed maximum growth were reversed with the two compounds, being $E/64$ for copper sulphate and nickel chloride, but $E/32$ for copper chloride and nickel sulphate. It is not possible, from the data available, to say if this difference is constant or really significant.

Cobalt chloride, like the sulphate, was the most toxic, $E/256$ being the greatest strength which permitted normal growth. At $E/8$ copper salts,

though markedly poisonous, permitted a fairly strong root growth, and even at four times the strength, with $E/2$, the root development with copper was better than with $E/8$ cobalt sulphate, in which practically no development of laterals had occurred (Pl. XXIV, figs. 5, 6). After the delayed appearance of signs of poisoning, the action was rapidly intensified, and the leaves were eventually much blotched and the roots unhealthy. With higher concentrations the bursting of the root cortex by the crowded and suppressed laterals was marked.

D. Discussion

In all work involving the association of toxic compounds and nutrient salts various factors are liable to influence comparisons. In earlier Rothamsted work (Brenchley, 1910, 1927) in which barley was grown with copper sulphate ($CuSO_4.5H_2O$) with and without nutrient salts, 1 p.p.m. of the sulphate alone sufficed to check all root growth. In the presence of nutrient salts, on the contrary, almost normal growth was made with this concentration, 1/100,000 being necessary to cause the same amount of damage, ten times as much as in the other test. There is always the possibility that more or less of the toxic substance may enter into insoluble combinations with some of the food salts and so be removed from the solution, thus weakening the concentration. With the nutrient solution used, such insoluble compounds would probably be phosphates. The proportion of potassium phosphate in the food salts is relatively heavy, about 0·5 g./l., whereas the quantity of toxic substance is small, the maximum amount of the poisonous element permitting normal growth in the present experiments ranging from 0·2–2 mg./l., according to circumstances, all greater amounts being extremely poisonous. If precipitation occurred, it might be expected that the overwhelming quantity of potassium phosphate could throw out much more of the toxic compound than was actually present, but the varying behaviour of the two species in identical solutions suggests that such precipitation probably does not occur here. Again, the fact that most "insoluble" substances are really very slightly soluble is of importance and, with the weaker concentrations at least, any phosphatic compound formed might be within the range of such solubility, thus leaving the whole of the poisonous compound in the solution. The degree of solubility would be affected by the *p*H of the nutrient solution.

From the practical and economic point of view, however, the important aspect of toxicity is in relation to plants growing with an adequate food supply, which may be presented normally in soil or

experimentally in nutrient culture. From this point of view the comparisons in the present series of experiments are valid, regardless of what reaction may take place between the poisonous and nutrient salts, since they indicate the trend of events under parallel conditions of plant growth. Jensen (1907) found that in solution cultures the lethal point of nickel nitrate for wheat plants was $n/2000$–$n/400$, but that by the addition of quartz to the solution the lethal limit was raised to $7n/2000$–$6n/4000$, a reduction of toxicity of six to seven times. In soil the toxic effect of poisonous compounds is usually much reduced, owing to adsorption by the solid particles, so that the level of dangerous concentrations may be considerably raised. In the Hill's pot culture experiments at Woburn, Voelcker (1913) found that wheat plants were damaged by copper sulphate supplying 0·05 % of copper reckoned on the weight of soil used: copper carbonate proved nearly as harmful, the nitrate was more toxic, whereas the insoluble phosphate could be used in much larger amounts without any injurious effects. These quantities are much larger than those which can safely be used in nutrient solution, showing that much of the toxic substance must have been inactivated by interaction with the soil.

It is generally recognized that all species do not respond to the same poison in the same way, but much of the work on the subject deals only with the early stages of growth and information covering the whole life cycle is comparatively scanty. In these experiments on cobalt, nickel and copper the general trend of response was much the same in all cases. Over a range of low concentrations growth was either similar to that of the controls or showed only a gradual depression. This was followed by a sudden check in development with the succeeding concentration, the point at which this occurred varying with the species and the toxic compound. The ultimate behaviour with greater strengths again varied.

With barley the general order of toxicity was copper > cobalt > nickel, though the difference was not very strongly marked and depended somewhat upon the compound used. With broad beans the greater toxicity of cobalt was outstanding, very little growth being made in comparatively low concentrations at which nickel and copper were inactive. Copper, on the other hand, was the least poisonous and, in strong concentrations, permitted an amount of growth that did not occur either with nickel or cobalt on beans, or with any of the three when applied to barley. The general order of toxicity with beans was cobalt > nickel > copper, the differences being greater than in the case of barley. Scharrer & Schropp (1933) carried out short-term experiments for about a fortnight with a variety of plants in culture solutions, and indicated that at low concen-

trations nickel was not so poisonous as cobalt, but at high concentrations there was little difference in their action. The latter statement, however, is now shown not to hold good consistently when plants are grown to maturity since, in Rothamsted tests, considerable variations in relative toxicity occurred in the higher concentrations. The greater tolerance of barley than beans to cobalt was shown in both sets of experiments. In earlier work by Coupin (1901) wheat seedlings were grown for twelve days in dilute solutions of the poisons dissolved in distilled water without nutrients. He found that, under these conditions, corresponding compounds of nickel and cobalt had very similar toxicities, the nitrates being much less toxic than the chlorides and sulphates. In India, Singh & Prasad (1936) found that wheat grown in Knop's solution exhibited slight toxicity with cobalt chloride at 0·0001 M ($=E/10$), the roots at 8 weeks being only 8·6 cm. long, compared with 22·6 cm. in the controls. At maturity the ears with cobalt ripened earlier, but the grain did not set well. This concentration is high compared with that permitting so much growth in barley, but the discrepancy may be due to the great difference in climatic conditions as well as to species response.

The general effect of the various sulphates and chlorides was not consistent, and no specific statement can be made as to which is the more toxic (Table VI). With cobalt the critical strength was identical in both the species tested. With copper the sulphate was more toxic than the chloride in both cases, and also was more poisonous to barley than to broad bean. With nickel, the relative toxicity was reversed both with the compounds and with the species, but as the concentrations were at such close range it is possible that the action was really similar, as in the case of cobalt.

Table VI. *Relative toxicities. Lowest concentrations which did not cause marked depression in growth*

		Barley	Broad bean
Sulphate	Cobalt	$E/64$	$E/256$
	Nickel	$E/64$	$E/32$
	Copper	$E/256$	$E/64$
Chloride	Cobalt	$E/64$	$E/256$
	Nickel	$E/32$	$E/64$
	Copper	$E/64$	$E/32$

The chlorides, acting on barley, provided the only instance in which very low concentrations caused a gradual depression of growth before the characteristic sudden drop. In other cases the same concentrations were inactive, giving plants as good as the controls. With higher strengths the barley died quickly, whereas beans made more growth. This may partly

be due to the different size of the seed, the amount of reserve food contained in the bean enabling the plant to make some growth in the presence of an amount of poison which prevents barley from utilizing inorganic food salts from the nutrient solution after its own small reserves are exhausted. Further work with other species of plants and under different conditions of growth is necessary to settle this point, and to determine the possible economic importance of nickel and cobalt when they occur together in the same soil.

E. SUMMARY

1. An account is given of the present position of our knowledge with regard to the distribution and the physiological importance of nickel and cobalt, in relation to plants and animals.

2. Experiments on barley and broad beans were carried out in water cultures with the sulphates and chlorides of cobalt, nickel and copper.

3. In every case a range of low concentrations did little or no damage, but toxic action occurred abruptly above a concentration which varied with the species and with the compound.

4. With barley, copper was the most poisonous element in either compound, but the differences were not striking. Low concentrations of the sulphate were innocuous, but parallel low strengths of the chloride caused a slight, significant depression in growth.

5. With broad beans, cobalt was much more poisonous than either nickel or copper, particularly with the sulphate. No slight depression with low concentrations of the chloride was noticeable with this species.

6. The morphological response to toxicity varied with the element concerned. Copper, in poisonous strengths, caused shortening and "bunching" of barley roots, whereas nickel and cobalt permitted the growth of elongated roots of a very attenuated nature.

7. The individuality of plant response to poison was frequently shown by the great variation in growth in the borderline concentrations just below those which caused marked depression of growth.

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EXPLANATION OF PLATES XXIII—XXV

PLATE XXIII

Fig. 1. Barley grown in nutrient solution with cobalt chloride. Concentrations: (*a*) control, (*b*) *E*/64, (*c*) *E*/16 (group of three), (*d*) *E*/4.

Fig. 2. Barley grown with nickel sulphate. Concentrations as fig. 1.

Fig. 3. Barley grown with copper sulphate. Concentrations as fig. 1.

PLATE XXIV

Figs. 1–4. Barley grown with nickel chloride, copper chloride and cobalt sulphate, in concentrations *E*/32, *E*/16, *E*/8, *E*/4 respectively. Photographed after 15 weeks' growth.

Fig. 5. Broad bean roots, showing greater toxicity of cobalt compared with copper. Concentrations, cobalt sulphate, (*a*) *E*/16, (*b*) *E*/8.

Fig. 6. Broad bean roots as fig. 5. Concentrations, copper chloride, (*a*) *E*/8, (*b*) *E*/4, (*c*) *E*/2.

PLATE XXV

Fig. 1. Broad beans grown in nutrient solution with cobalt sulphate. Concentrations: (*a*) control, (*b*) *E*/512, (*c*) *E*/256, (*d*) *E*/128, (*e*) *E*/64, (*f*) *E*/32, (*g*) *E*/16, (*h*) *E*/8, (*k*) *E*/4, (*l*) *E*/2.

Fig. 2. Broad beans with nickel chloride. Concentrations as fig. 1.

Fig. 3. Broad beans grown with copper chloride. Concentrations as fig. 1, with addition of (*m*) *E*.

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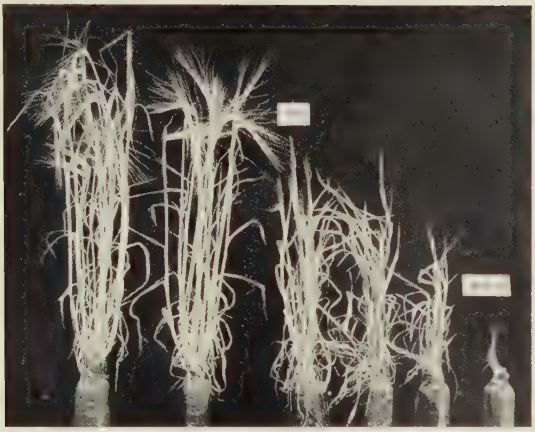

Fig. 1.

Fig. 2.

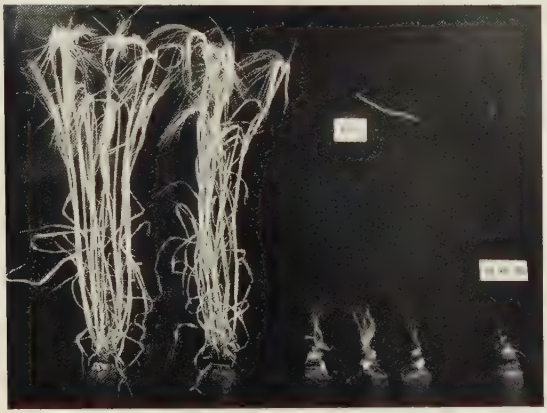

Fig. 3.

Fig. 1.

Fig. 2.

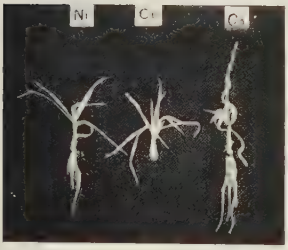

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

BRENCHLEY.—Comparative Effects of Cobalt, Nickel and Copper on Plant Growth (pp. 671–694).

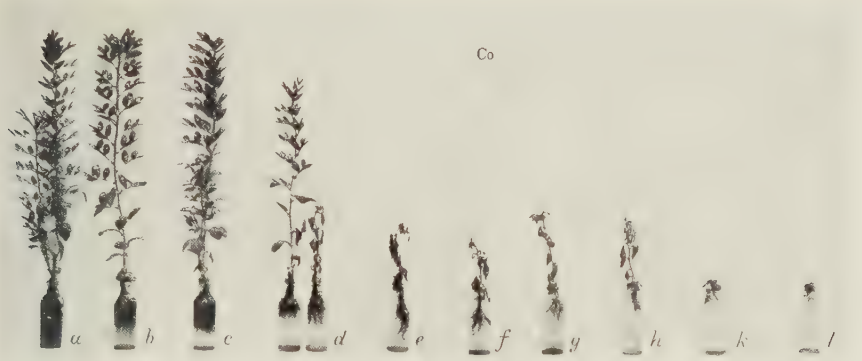

Fig. 1.

Fig. 2.

Fig. 3.

BRENCHLEY.—Comparative Effects of Cobalt, Nickel and Copper on Plant Growth (pp. 671–694).

CROSS- AND SELF-FERTILITY IN SAINFOIN

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Introduction

An essential preliminary to any scheme of crop improvement or seed production is to find out to what extent the particular species under consideration is capable of setting seed by self-pollination. This knowledge will determine the breeding methods to be used and the precautions necessary to maintain the purity of stocks. In the case of leguminous herbage plants the problem has been investigated in numerous species. Williams (1931 *a*, *b*, *c*, *d*) has examined species of *Trifolium*, *Medicago* and *Anthyllis* at Aberystwyth and has classified them into three categories: (1) voluntarily self-fertile species, i.e. species which are able to effect self-pollination and fertilization without the aid of external agents; (2) species which are practically incapable of effecting self-pollination without the aid of external agents, but which are fully self-fertile when artificially self-pollinated; and (3) species which are almost completely self-sterile even when artificially self-pollinated.

As regards sainfoin, Fleischmann (1932), in Hungary, investigated seed setting in Persian sainfoin (*Onobrychis viciaefolia* var. *persica* Sir.) and found that racemes set an average of 25·5 seeds when left open, though there was considerable variation in the actual numbers. When the racemes were bagged, one quarter of them set no seed, while the remainder

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set from one to nine, the average number of seeds per raceme being 1·5. An account of the pollination mechanism has been given by Müller (1883). In the present work the relative importance of cross- and self-pollination in the varieties of sainfoin grown in England (*O. sativa* Lam. = *O. viciaefolia* var. *sativa* Lam.) has been examined.

Materials

The work was done at the Agricultural Botanic Garden of the University of Reading. Seeds of the Giant and Common varieties of sainfoin were sown in drills in the spring of 1935. Giant sainfoin flowers in its seeding year and, in subsequent years, flowers two or three times so that two or three cuts can be taken. Common sainfoin does not flower in its seeding year, and in subsequent years flowers only once, providing only one hay cut followed by grazing. The investigations were made in May and June 1936 and 1937. Seeds of giant sainfoin are harvested normally after the second flowering, but these experiments were carried out at the first flowering in order to give a more accurate comparison with common sainfoin which flowers once only.

Pollination

The inflorescence of sainfoin is a dense spike-like indefinite raceme borne on a long axillary peduncle. The flower has a very short pedicel and is subtended by a small subulate bract, longer than the pedicel. The calyx is pubescent and has five long pointed teeth and a short tube. The polypetalous corolla is pink with red veins. The standard is notched at the apex and the limb is slightly turned up. The keel is almost as long as the standard, its claws are short and its limbs transversely truncate. The wings are half-sagittate, very small and completely hidden. The stamens are fused for two-thirds of their length, except the posterior one which is free and shorter than the others. The ovary is ovoid and pubescent, containing a single ovule and is borne on a short gynophore. The style is long and the terminal third is bent up at right angles and projects beyond the anthers. At the apex of the raceme is a tuft of undeveloped flowers. The flowers open from the base of the raceme upwards and a period of ten to twelve days elapses between the opening of the first flower and the withering of the terminal flowers.

In consequence of the small size of the wings, the full weight of a visiting insect is taken by the keel. When this is depressed the stigma and anthers come into contact with the insect's body and, when it is released, the keel returns to its original position. A flower may therefore be tripped

by an insect several times. According to Knuth (1906), the style becomes more erect as anthesis progresses, ultimately projecting 1–1·5 mm. from the keel cleft, thus excluding automatic self-pollination and ensuring cross-pollination.

As the calyx tube is short and the petals completely free, the nectar is easy of access and a variety of insect visitors might be expected. Knuth (1906) lists 52 insects which have been observed, mostly by Müller, to visit sainfoin in Central Europe. This list is made up of 41 Hymenoptera, one Dipteron and ten Lepidoptera. According to Müller's observations (1883), the honey bee is by far the most frequent visitor, paying nine out of every ten visits recorded. At Shinfield the most frequent visitor was the honey bee; other insects observed were humble bees and various Lepidoptera. Darwin (1878) records an occasion when a field of sainfoin was cut and he observed honey bees "driven to desperation" attempting to obtain nectar from red clover in an adjoining field.

Number of flowers per raceme

On the stem of sainfoin, racemes arise in succession from the lower nodes upwards and as many as five or six may be produced. The numbers of flowers in the three lowermost racemes were counted. On 27 May 1937 the lowermost raceme was taken for counting from one stem of each of 50 common sainfoin plants chosen at random. Similarly, 50 racemes of giant sainfoin were taken on 29 May. When the second and third racemes came into flower, 50 each of giant and common sainfoin were removed for counting. There was, therefore, a set of 100 of each of the first three

Table 1

No. of flowers per raceme	% frequency	
	Giant	Common
10–19	2·7	0·7
20–29	2·7	4·0
30–39	16·0	10·0
40–49	26·0	17·3
50–59	20·0	18·7
60–69	19·3	20·7
70–79	6·7	16·7
80–89	3·3	6·7
90–99	2·0	4·7
100–109	0·7	0·7
110–119	—	—
120–129	0·7	—
No. of racemes counted	150	150
Mean	52·7	58·9
S.E.	1·42	1·45
Average of giant and common	55·8	

racemes, one half being from giant and one half from common sainfoin. The three sets were not necessarily taken from the same stems or from the same plants.

The counts are given in Table I in which those from the three sets of racemes are combined. Considerable variation was found in the number of flowers per raceme. The number ranged from 15 to 127, but the majority of racemes contained between 30 and 80. The mean number of flowers per raceme was found to be 58·9 in common and 52·7 in giant sainfoin, the difference being significant. Taking the giant and common varieties together, the mean number was 55·8.

The figures were analysed to discover if there was any difference in the number of flowers per raceme at different nodes. Combining the figures for the two varieties, the numbers of flowers were:

1st raceme – 58·4 ±2·00
2nd „ – 56·3 ±1·50
3rd „ – 52·7 ±1·77

These figures appear to indicate that the later formed racemes bear fewer flowers than the earlier ones, but the differences, even between the first and third, are not significant.

Fruit set

The numbers of fruits per raceme were counted in the same way as the numbers of flowers. The results are given in Table II and show considerable variation in the number of fruits set. The number of fruits per raceme ranged from 1 to 72, but the majority of racemes bore between 10 and 50. In giant sainfoin the mean number of fruits per raceme was 29·37 and in common sainfoin 31·03, but the difference is not significant. Taking the two varieties together, the mean number of fruits was 30·2.

Table II

No. of fruits per raceme	% frequency	
	Giant	Common
0–9	9·3	1·3
10–19	17·3	14·7
20–29	28·7	35·3
30–39	20·7	30·0
40–49	13·3	9·3
50–59	6·7	4·0
60–69	2·7	4·7
70–79	1·3	0·7
No. of racemes counted	150	150
Mean	29·37	31·03
S.E.	1·245	1·045
Fruit set	55·7%	52·7%
Average fruit set of giant and common	54·1%	

The "fruit set" was obtained by expressing these figures as percentages of the number of flowers per raceme (Table I). In giant sainfoin the fruit set was 55·7% and in common sainfoin 52·7%, there being no significant difference, and the average of the two varieties was 54·1%. Thus little more than half the flowers set seed.

The numbers of fruits set at different nodes were found to be as follows:

	No. of fruits	Fruit set
1st raceme	28·6 ± 1·45	50·0%
2nd ,,	33·9 ± 1·57	60·2%
3rd ,,	28·1 ± 1·08	53·3%

The second raceme had a higher fruit set than the first or third but the difference is not significant.

SELF-FERTILITY

The extent to which sainfoin is capable of setting seed by self-pollination was studied in 1936 and 1937. Thirty-six giant plants and 39 common plants were used. On each plant three racemes with unopened buds were chosen. Two of these were enclosed in wax paper bags plugged with cotton-wool and the third was left as a control. One of the bagged racemes was left untouched until the fruits had set. The other had its bag removed every day while in flower, the open flowers were tripped with a sterile blunt needle and the bag replaced. The only chance of cross-pollination was from wind-borne pollen during the tripping process, but as this took only about a minute, there was little possibility of this happening. When the fruits had set and had developed sufficiently to ensure that they each contained a seed, the bags were removed and the fruits counted (Table III).

When the flowers were tripped 20 racemes set no fruits, and the number of fruits in the remaining 55 racemes varied from 1 to 17. The mean number of fruits set per raceme with tripped flowers was 2·853. When the flowers were left untouched, 53 racemes set no seed and, in the remaining 22 racemes, the number of fruits set varied from one to four. The mean number of fruits set per raceme with untripped flowers was 0·547. The difference between the fruit-sets of tripped and untripped racemes is significant. The controls, which were open to free pollination by bees set 28·767 fruits per raceme. The fertility was calculated by expressing these figures as percentages of 55·8, the mean number of flowers per raceme (Table I). The fertilities were 5·11, 0·98 and 51·55% for tripped, untripped, and control racemes respectively.

That the degree of self-fertility in sainfoin is very low was confirmed by other observations made in 1937. Five 2-year old plants were growing

Table III

No. of fruits per raceme	Frequency		
	Flowers tripped	Flowers not tripped	Controls
0	20	53	—
1	14	12	—
2	12	4	—
3	7	3	—
4	6	3	—
5	4	—	—
6	2	—	1
7	1	—	—
8	2	—	—
9	2	—	—
10	3	—	1
11	1	—	1
12	—	—	2
13	—	—	2
14	—	—	1
15	—	—	1
16	—	—	2
17	1	—	5
18	—	—	2
19	—	—	2
20–29	—	—	22
30–39	—	—	20
40–49	—	—	9
50–59	—	—	2
60–69	—	—	1
70–79	—	—	1
No. of racemes counted	75	75	75
Mean	2·853	0·547	28·767
S.E.	0·3845	0·1196	1·4480
Fertility	5·11%	0·98%	51·55%

in pots in a greenhouse. The pots were standing together and no attempt was made to protect the flowers against cross-pollination, but no bees were ever observed in the house. These five plants produced 231 racemes, and 217 of these produced no fruits. Of the remainder, ten set one fruit each, two set two fruits each, one set three fruits and one set four fruits, the average for the 231 racemes being 0·09.

The figures were analysed to ascertain if there was any difference between the two varieties or between the two years in which the experiments were carried out.

Taking the results of 1936 and 1937 together, the numbers of fruits per raceme were:

Flowers tripped:	Giant	$2{\cdot}472 \pm 0{\cdot}4992$
	Common	$3{\cdot}205 \pm 0{\cdot}5726$
Flowers untripped:	Giant	$0{\cdot}556 \pm 0{\cdot}1592$
	Common	$0{\cdot}538 \pm 0{\cdot}1771$

These slight differences between giant and common sainfoin are not significant.

Taking the giant and common varieties together, the numbers of fruits per raceme were:

Flowers tripped:	1936	$2{\cdot}961 \pm 0{\cdot}5189$
	1937	$2{\cdot}167 \pm 0{\cdot}5609$
Flowers untripped:	1936	$0{\cdot}628 \pm 0{\cdot}1491$
	1937	$0{\cdot}50 \pm 0{\cdot}2205$

The differences between the two years are not significant.

Seedlings from selfed and crossed seeds

Seeds obtained by self-fertilization in 1936 were sown in the open in 1937 and the seedlings kept under observation. Out of 47 selfed seeds from giant sainfoin only 5 gave established seedlings, and out of 45 selfed common seeds 18 gave established seedlings. The seedlings appeared to be normal morphologically, but in some growth was very slow. Growth was very poor in the five giant seedlings, though one produced flowers. Of the 18 common seedlings growth was recorded as unsatisfactory in five. These observations are not conclusive, but they suggest that Common is more likely than giant sainfoin to give vigorous seedlings from selfed seed.

Seeds from the control racemes were sown for comparison with the selfed seeds. No establishment values were taken but, in comparison with the seedlings from selfed seeds, practically all the established seedlings grew vigorously. Of 31 giant seedlings 25 flowered, and of 34 common seedlings none flowered.

Discussion

The difference between the giant and common varieties in the number of flowers per raceme should be considered in conjunction with differences in the vegetative characters of the seedlings. It has been shown (Thomson, 1938) that the leaves of giant sainfoin seedlings tend to have a greater number of leaflets than the leaves of common sainfoin seedlings. In flower production, however, the position is reversed, common sainfoin having a greater number of flowers per raceme than giant sainfoin.

Out of an average of 55·8 flowers per raceme, little more than one half set seed under normal conditions of open pollination. The number of fruits set per raceme, 30·2, was rather higher than the figure given by Fleischmann (1932) for Persian sainfoin growing in Hungary. The proportion of

flowers setting seed was of the same order as that found for red clover by Williams (1931*a*). There is no evidence to suggest a possible reason for this low fertility. According to Williams (1927) the number of bees may be a limiting factor in the seed production of red clover, and this may also apply to sainfoin, but there appeared to be no shortage of honey bees on the plots.

The maximum self-fertility possible when every stigma is dusted with its own pollen by artificial tripping is 5·11%. In the absence of tripping agents the fertility is 0·98%. It appears, therefore, that sainfoin is slightly self-fertile but is usually cross-pollinated. The reduction of fruit-set from 5·11 to 0·98% when the flowers are not tripped shows that the mechanism described by Knuth (1906) as preventing automatic self-pollination is fairly effective. The number of fruits set per raceme when the flowers were untouched was lower than that found by Fleischmann (1932) in Persian sainfoin but, as he does not state the number of flowers per raceme, it is impossible to say whether Persian sainfoin shows any significant difference in self-fertility. Reference has been made above to Williams's classification of herbage legumes according to their self-fertility. Sainfoin fits into none of his three categories, but is intermediate between the second and third, being almost incapable of pollinating itself but slightly self-fertile when artificially self-pollinated. The self-fertility was very low when the plants were grown under glass and no difference was found between the two seasons 1936 and 1937. This indicates that self-fertility is uniformly low and is not greatly influenced by environmental conditions as in Lucerne (Williams, 1931*c*). Any scheme of improvement in sainfoin must therefore depend on building up families and not on selection of single plants and subsequent self-pollination.

The reduction in vigour of the selfed seedlings is what might be expected, though there were no chlorophyll-deficient seedlings such as occur in red clover (Williams, 1931*a*).

Rees (1928) found that some lots of common sainfoin flowered in the seeding year. Certain regional strains of common sainfoin are intermediate between the giant and the true common variety (Thomson, 1938), but Rees has pointed out one possible reason for seeding year flowering which may be mentioned, namely that the parent common sainfoin plants were fertilized by pollen from neighbouring fields of giant sainfoin. That this is highly improbable is shown by the behaviour described above of common seedlings grown from cross-pollinated seeds. The plants from which the seeds were taken were growing in two plots 40×9 ft. and separated from similar plots of giant sainfoin by only 6 ft.

Both varieties were in flower at the same time and there was, therefore, ample opportunity for hybridization, yet none of the next generation seedlings showed any flowering. There are three possible explanations of this:

(*a*) Giant and common sainfoin are intersterile. There is, as yet, no evidence on this point.

(*b*) The F_1 generation of a common × giant cross does not flower in its seeding year. There is, as yet, no evidence on this point.

(*c*) Hybridization did not take place.

Whichever be the true explanation, seeding year flowering in common sainfoin cannot be due to fertilization of the parent by giant pollen. The third possibility seems the most probable and is supported by the behaviour of the visiting insects. If flowers are abundant honey bees tend to work within a very small area and do not fly any distance from one plant to the next.

In the case of giant sainfoin the possibility of hybridization does not arise as seed is harvested after the second flowering and there is no chance of pollination from common sainfoin.

Summary

1. The number of flowers per raceme is slightly greater in Common than in giant sainfoin.

2. Little more than half the flowers set seed under conditions of open pollination.

3. When the flowers were protected and left untouched the self-fertility was found to be 0·98%. When the flowers were artificially self-pollinated by tripping the self-fertility was significantly higher, being 5·11%.

4. Seedlings grown from selfed seed showed poor establishment and reduced vigour.

5. Evidence is brought forward which suggests that the phenomenon of seeding year flowering in common sainfoin cannot be due to the fertilization of the parent plants by pollen from giant sainfoin.

Thanks are due to the following seeds merchants who supplied samples of seed: Dunn's Farm Seeds, Ltd., Salisbury; Gartons, Ltd., Warrington; A. G. Leighton, Ltd., Whitchurch; Sutton and Sons, Ltd., Reading.

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ROOT ROT, SHOOT ROT AND SHANKING OF TULIP CAUSED BY *PHYTOPHTHORA CRYPTOGEA* PETHYBR. & LAFF. AND *P. ERYTHROSEPTICA* PETHYBR.

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(With Plates XXVI and XXVII)

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Introduction

These investigations began with the receipt of information in February 1930 from the Plant Pathological Laboratory of the Ministry of Agriculture at Harpenden that specimens of forced tulips submitted from a grower in Hampshire had been diagnosed as attacked by *Phytophthora cryptogea*. Twelve thousand out of 30,000 bulbs of the variety William Copland had failed to flower, while a large batch of variety Bartigon then being forced was unsatisfactory. The bulbs had been planted in rather heavy soil in poorly drained boxes, and the trouble had been attributed by another consultant mainly to those factors. Slugs and other pests had been troublesome, but it was possible readily to isolate a species of

Phytophthora in pure culture from some of the specimens, as well as from a few of the Wm Copland plants which had been preserved.

The first record of this disease, for which the name "Shanking" was suggested at the time, had been made by the Plant Pathological Laboratory in February 1928 (Pethybridge *et al.* 1934). Specimens of the variety Vermilion Brilliant received from Middlesex showed contraction, wrinkling and ultimate death of the bases of the flower stalks, so that the flowers failed to develop beyond the bud stage (Pl. XXVI, fig. 1). From the affected tissues *P. cryptogea* was isolated, and the identification was confirmed by Ashby (1929*b*). During the early months of 1928, and of 1929, further similar specimens were received at Harpenden from Lancashire, Scotland and other parts of the country. The variety most frequently affected was Wm Copland, but the disease was also seen in Wm Pitt, Bartigon and Tea Rose. A short account of the disease was published by Foister (1930) and, apart from a brief reference to its occurrence in the Spalding area by E. R. Wallace (1936), there is no further literature on the subject. When these researches were begun little or nothing was known about the disease. It was assumed that infection took place from the soil via the roots and that *P. cryptogea* was the cause, but no definite evidence in support of either assumption had been obtained.

During the course of this work over 10,000 experimental bulbs have been grown, and individually recorded, while observations have been made on material obtained from commercial nurseries. Shanking represents only one phase of the disease, and all stages of attack may be obtained from that of a bulb which fails to give rise to any shoot above the neck of the bulb, through that of shoots which rot off at the base before the leaves unfold, to that of typically "shanked" flowers, and even to examples where a marketable flower, probably of poor quality, is cut, although the plant contains the parasite in its roots. Further, the disease has been induced for the first time in outdoor plantings of tulips.

The experiments carried out proved not only that *P. cryptogea*, but also *P. erythroseptica*, may be the cause of the disease. Apart from obtaining negative results from a few trials with *P. cryptogea* var. *Richardiae*, and with *P. parasitica*, no attempt has been made to deal exhaustively with other species of *Phytophthora* as possible pathogens of the tulip. From time to time during the past 8 years *Pythium* spp. (Moore & Buddin, 1937) have been isolated from diseased tulips exhibiting symptoms not unlike the present disease. In the larger commercial nurseries in the Southern Advisory Province, however, the *Pythium* disease

appears to be of little importance compared with that due to *Phytophthora* spp.

It has been shown that the soil is the outstanding source of infection on the nursery. Effective means of control have been demonstrated, and the importance of putting these into practice is evident when it is realized that not infrequently houses, containing 30,000 bulbs which have been forced, produce not a single marketable flower.

Phenomena that might be confused with true Shanking. Blindness in tulips may result from too early forcing, from wide fluctuations in temperature, or too high a temperature during the forcing period, especially with "prepared" bulbs, or from forcing too small a bulb, especially if it has been badly stored, or has been damaged by *Penicillium*. Alternatively, a very mild attack of Shanking may give the grower the impression wrongly that he has been supplied with bulbs which were too small for forcing, or that they came from a "weak stock".

The flower buds may also fail occasionally through allowing the plants, or the atmosphere, to get too dry at a critical stage, while "pseudo-shanking" may result from the careless use of fertilizers.

METHODS

Isolation of the parasites. The great majority of the isolations were made by immersing cut portions of the infected material in a shallow layer of water in deep Petri dishes, which could be incubated and dealt with in large numbers. This method was particularly useful for the examination of roots, and permitted frequent observation of the material with the low power of the microscope without serious risk of contamination. Later, transfers were made from the mycelium in the water cultures to set plates of either Waksman's acid peptone agar, or of Quaker Oat agar, any excess of water having previously been drained off. At 25° C. the *Phytophthora* usually grew away readily from contaminating bacteria, etc., but sometimes transfers were made to further set plates of Quaker Oat agar before the pure cultures were tubed.

With material in an advanced stage of attack, it is usually easier to demonstrate the presence of the parasites in the roots, if any remain, than in the rotting base of the stem, owing to the presence of numerous secondary organisms in the latter.

Contamination of the soil. In pathogenicity tests, new sterilized boxes, constructed with adequate drainage, were filled with steamed soil. After a suitable interval the top layer of soil was removed and pure cultures of the strains were mixed with the soil that was to lie immediately below the bases of the bulbs. The bulbs were then planted and covered with the soil that had been temporarily removed. Liberal, approximately equal, amounts of inoculum (usually the chopped contents of 6–12 tube cultures on Quaker Oat agar to each box) were used.

Owing to the frequency of mixed infections with *Phytophthora* spp., and the likelihood of introducing *Sclerotium Tuliparum*, or *Botrytis Tulipae*, from commercial establishments, it was only rarely that contaminated soil from nurseries was used. In

testing "over-summering" of the parasites, and methods of control, soil which had been contaminated with pure cultures for one or more years was used. There was some variation in the size of boxes used and, consequently, in the number of bulbs planted in them but, throughout the experiments, the degree of crowding of the plants was comparable with ordinary commercial conditions. No difficulty was experienced in forcing successive crops of tulips in the soil.

The experimental bulbs. When necessary "own grown" bulbs were investigated but, ordinarily, imported tulip bulbs were used. The bases of the old flowering stalks, and the brown outer tunics, were removed to minimize complications from tulip fire, and to enable more careful examination for their freedom from minor troubles, such as lesions caused by *Penicillium.* No form of surface sterilization of the bulbs was attempted, but no serious complications arose from diseases that might have been carried with the bulbs. Occasionally, a shoot was infected with *Fusarium avenaceum* (Beaumont & Buddin, 1938), but fire (*Botrytis Tulipae*) occurred only to a very slight extent under glass, although it was troublesome in the tulips grown in succession out of doors. Clear-cut results were prevented in some instances by the difficulty of maintaining a uniform temperature for flowering in part of the available glasshouse. The optimum temperature for these species of *Phytophthora* is 77–81·5° F., so that a satisfactory forcing temperature of 65–70° F. would have favoured the parasites at least as much as the tulips. When the temperature could be maintained, first-class flowers were obtained as early, and as fine in quality, as those commonly marketed. It is of interest to mention the statement by Mr Geo. Shawyer (1932) that, even on his nurseries, the average waste in tulips, i.e. the actual difference between the number of blooms sold and the number of bulbs bought, was 9·12% over a period of 10 years.

The pre-forcing period. The bulbs were planted in September, and then placed in a partly shaded position, bedded in, and covered by several inches of well-washed and sieved, sterilized ashes. Spaces approximately 2 in. wide, packed with ashes, were left around individual boxes, and there was no evidence of any spread of infection from one box to another during the 2–3 months in which the bulbs were forming their root systems out of doors.

Presentation of data. A complete picture of the results of any particular experiment would require a mass of statistical data showing the amount of growth at various periods during forcing. This would be meaningless without careful standardization of the types, and weights, of the planted bulbs, and greater control of watering and temperatures in all parts of the forcing house than was possible. An "adequate" number of bulbs was always employed and, as the production of marketable blooms was considered to be the most important practical criterion, most of the results, other than descriptive ones, are presented in that form. Perhaps the greatest need for some amplification of the figures arises in connexion with Table III; in the three instances where no flowers of either variety were produced the growth of the Wm Pitt plants during the first weeks of forcing was definitely better than that of the Wm Copland. On the other hand with strain 21 of *P. cryptogea* in 1931–2 the Wm Copland plants made good foliage growth in spite of yielding only two blooms, while most of the twenty flowers of Wm Pitt were below first quality, and some of the roots were attacked. All the important experiments were replicated, and no emphasis was placed on any but obviously significant differences in numbers of flowers, after considering the growing conditions.

EXPERIMENTS WITH VARIOUS SPECIES AND STRAINS OF *PHYTOPHTHORA*

The fungus first isolated from "shanked" tulips at Harpenden in 1928 was identified as *Phytophthora cryptogea* Pethybr. & Laff. Early in 1930, amphigynous oogonia were produced in abundance on the Hampshire material, and isolations from different plants had all produced numerous oogonia on Quaker Oat agar by the following September, when one of the isolates was submitted to Mr S. F. Ashby of the Imperial Mycological Institute. The oospores were larger than those of the 1928 tulip strain of *P. cryptogea*, having a range on Quaker Oat agar from 18 to 42 μ, with an average diameter of 30·5 μ, while the oogonia ranged from 23 to 46 μ, with a mean diameter of 35 μ. The fungus was provisionally identified as either *P. cryptogea* var. *Richardiae* (Buism.) Ashby (Ashby, 1929*b*, p. 259), or *P. erythroseptica* Pethybr. (Pethybridge, 1913)[1] but the final decision was difficult, owing to different measurements having been recorded by different authors for the oospores of *P. erythroseptica*. The original material had conidia of the non-papillate type with a shallow apical, hyaline layer. Ashby measured conidia growing in a water culture at 23° C. from a young growth on bean agar—a medium giving larger and more abundant conidia than oat agar. On the fourth day twelve of the larger, fully mature, conidia—some of which showed internal cleavage into zoospores—yielded a mean of 43·5 × 27·5 μ (length/breadth ratio = 1·58), and a range of 36–55 × 21–33·5 μ. The facile production of both sexual organs and conidia suggested *P. erythroseptica*.

In order to determine the identity of the parasite concerned inoculations were made with named species of *Phytophthora*, and further isolations were made from plants from commercial nurseries. Five strains of *P. erythroseptica* and two of *P. cryptogea* var. *Richardiae* were obtained through the courtesy of the Centraalbureau voor Schimmelcultures and of the Lister Institute. On maize-meal extract agar the Hampshire strain was indistinguishable in colony texture and hyphal character from the *erythroseptica* strains which, apart from some differences in growth rate, were similar to one another. On the other hand, the two *Richardiae* strains resembled one another but were unlike the others. This evidence

[1] Later, in February 1932, Ashby considered the strains used in these experiments in the light of *P. Drechsleri*, which had recently been described by Tucker (1931). The latter grows freely at 30° C., and will grow even at 35° C. on maize agar plates, but the tulip strain corresponded to *P. erythroseptica* (and *P. cryptogea* var. *Richardiae*) in that there was no continued growth at 30° C.

strengthened the indication that the Hampshire strain from tulips was *P. erythroseptica.*

A. The following authentic strains of *P. erythroseptica* were used in 1931–32:

(1) Isolated from Hampshire (1930) tulip material, and used by S. F. Ashby for comparative cultural work.

(2) Parallel culture to no 1, but kept continuously at Reading.

(3) Isolated from potato by G. H. Pethybridge, and originally received at the C.B.S. in January 1923.

(4) A later isolation from potato by G. H. Pethybridge, received at the C.B.S. in December 1929.

(5) Isolated from *Atropa* by Mrs N. L. Alcock (1926), and sent to S. F. Ashby by C. E. Foister.

(6) Isolated from potato by A. E. Muskett, and received by the C.B.S. in March 1930. (Found by Ashby to resemble no. 1 particularly closely.)

(7) The same strain as no. 4, but passed through potato tubers and reisolated in December 1930.

In addition, isolates numbered 8–16 that had been identified by the writer were used. They had originated from the Hampshire material; no. 14 had been in culture from the spring of 1930, while nos. 8, 9, 11, 13 and 16 had been reisolated from either Wm Copland or Wm Pitt bulbs grown indoors or out of doors in soil contaminated with these isolates during 1930–1. Nos. 10 and 15 had been isolated from bulbs adjacent to inoculated bulbs, while no. 12 was isolated from a Wm Pitt bulb grown in soil contaminated with a portion of the infested nursery soil.

The results obtained when these strains were used to contaminate the soil are given in Table I. The presence of *P. erythroseptica* was demonstrated in diseased plants from almost every box, and pure cultures were made from the majority. These cultures were used for further soil contamination, and the parasite was repeatedly reisolated. Mr Ashby examined sporangia and oospores developing from stumps of typical Wm Copland plants infected with strains 1, 4, 6 and 7: the sporangia were variable in size and non-papillate, while the oospores were always amphi-

Table I. *Numbers of marketable flowers produced in soil contaminated with the strains of* P. erythroseptica *indicated. Normally, twenty bulbs were planted in each box, and the Wm Pitt were forced several weeks later than most of the Wm Copland*

	Clean soil controls		Strain of *P. erythroseptica*														
Variety	A	B	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Wm Copland	18	17	1	5*	10	1	0**	0	2	0	0	4	2	2	1	3	19	13
Wm Pitt	20	19	1	0*	20	0	4	0	1	0	2	0	0	0	0	2	3	0

* Twenty-five bulbs per box. ** The plants in this box made very fair foliage growth, but all failed to flower owing to the disease.

gynous, and from 28 to 33 μ in diameter, with oogonia of 33–38 μ, although diminutive sizes could be found. Measurements of reproductive bodies developing on the host material from which strain 8 was isolated were: sporangia $49 \times 30\,\mu$, with a range of 35 69×19 $36\,\mu$ and the high length breadth ratio of over 1·6. Oospores 25–29 μ with walls about 3 μ thick; oogonia 30–37 μ with amphigynous antheridia.

The comparatively free flowering of the Wm Copland plants in soil contaminated with strains 15 and 16 may have been due to a small quantity of inoculum, or to some unrecorded difference in the conditions. The results with strain 3 are interesting. Although all twenty of the Wm Pitt bulbs flowered successfully, sporangia of *P. erythroseptica* were obtained readily from the roots which were examined after the flowers had been cut. This strain had been *in vitro* for some 8 years; it differed from the other strains in spreading on maize agar plates more rapidly, and in forming no sexual organs on Quaker Oat agar under conditions in which the other strains produced them freely. The reaction of this strain at 30° C. was also rather different: during the first 3 days after transfer from 23° C. on maize agar the diameter of the colony increased three times as much as the other strains, although none of them continued growth at 30° C. between the third and fifth days. In dealing with *P. palmivora* Ashby (1929*a*) mentioned instances of loss of or decreased pathogenicity of strains in artificial culture for several years, while Thompson (1929) recorded that a strain of *P. Colocasiae* failed to infect after 2 years in culture although, when freshly isolated, it caused a patch canker of *Hevea* rubber.

Notwithstanding these minor irregularities, consideration of Table I, and the growth of the plants throughout the season, can leave no doubt about the pathogenicity to the tulip of authentic strains (4, 6 (Pl. XXVI, fig. 2) and 7) of *P. erythroseptica* originally obtained from potatoes, of the numerous isolates and reisolates from the Hampshire material, or even of strain 5 from *Atropa*. This was thought by Alcock (1926) to be a distinct variety, although Tucker (1931) stated that "the reasons for separating the *Atropa* strain are not very convincing, and, pending further evidence, the writer prefers to include it in *P. erythroseptica*".

B. The following strains of *P. cryptogea* var. *Richardiae* were used in 1931–2:

(17) Received from the Lister Institute.

(18) From the C.B.S. Originally received at Baarn in February 1927, having been isolated about 1926. In 1932–3 tests were made with strain 19, isolated by Miss Buisman from Calla Lily in 1930: received from the C.B.S. February 1932.

The results obtained when these strains were used for soil contamination are shown in Table II. Owing to the poor cultural conditions for the plants in these particular boxes the results in 1931–2 were not satisfactory, but no evidence was obtained that the failures were due to parasitic activity of the fungus. The results with the more recently isolated strain used in 1932–3 were satisfactory. Although more work needs to be carried out with the *Richardiae* variety of *P. cryptogea*, it appears to be either not pathogenic to forced tulips, or is of no more importance than species of *Pythium* (Moore & Buddin, 1937). Considering the above results in conjunction with those in the preceding section, it seems clear that the parasite concerned in the Hampshire nursery in the spring of 1930 was *P. erythroseptica*.[1]

Table II. *Numbers of marketable flowers produced in soil contaminated with strains of* P. cryptogea *var.* Richardiae. 20 *bulbs per box*

	Season 1931–2				Season 1932–3	
	Controls in clean soil		Strain of *P. cryptogea* var. *Richardiae*		Controls in clean soil	Strain of *P. cryptogea* var. *Richardiae*
Variety	A	B	17	18		19
Wm Copland	18	17	11	16	20**	20
Wm Pitt	20	19	20	7*	19***	20

* Forced early in an inadequately heated house with large fluctuations in temperature. A comparable box of the same variety in *clean* soil gave only 11 flowers. ** Average of four boxes. *** Average of two boxes.

C. *Soil contamination with* P. parasitica *Dastur.* A strain of *P. parasitica* was isolated from tomato plants affected with foot rot in August 1935, and was used in the autumn to contaminate duplicate boxes of sterilized soil. Twenty-five Wm Copland tulips were forced in each box, and were then placed out of doors until the following autumn when the new bulblets, which appeared to be quite healthy, were removed. New Wm Copland bulbs were forced in the same soil during the following season. In 1935–6 forty-nine flowers and, in 1936–7, forty-seven flowers were cut from plants growing in contaminated soil, while twice the number of bulbs in clean soil gave ninety-nine and ninety-three respectively. From this small amount of evidence there is no reason for suspecting *P. parasitica* to be a root parasite of forced tulips.

[1] To avoid misunderstanding it may be stated here that the name *P. cryptogea* var. *Richardiae* was used in error instead of *P. erythroseptica* on p. 287 of *Reports on the work of Agricultural Research Institutes and on certain other agricultural investigations in the United Kingdom for* 1930–1, published by the Ministry of Agriculture in 1932.

D. *Soil contamination with* P. cryptogea *Pethybr. & Laff.* As the fungus first isolated from "shanked" tulips was *P. cryptogea*, and this species has also been isolated many times since from nurseries, experiments were set up to test an authentic strain of it alongside strains isolated from tulips: at the same time its effects were compared with those of *P. erythroseptica*. The following strains were used in 1931–2:

(21) Isolated from aster by L. Ogilvie, and *in vitro* for a few years.

(22) Isolated in spring 1931 from tulips on a commercial nursery in Middlesex.

In 1932–3 strains 21 R and 22 R, reisolated from the plants attacked the previous year, were used together with the following additional strains:

(23) Isolated in spring 1932 from tulips in Middlesex.

(24) Isolated from tomato plant affected with foot rot.

Strains 22 and 22 R were compared with a culture of *P. cryptogea* from Baarn, and were found to be comparable, giving non-papillate, proliferating sporangia with a length/breadth ratio of about 1·45.

The results obtained from soils contaminated with these strains are given in Table III (see also p. 708).

Table III. *Numbers of marketable flowers produced in soil contaminated with strains of* P. cryptogea. *Twenty bulbs per box, the Wm Pitt being forced after the Wm Copland*

	Season 1931–2				Season 1932–3						
	Clean soil controls		Strain of *P. cryptogea*			Strain of *P. cryptogea*					
							22 R			24	
Variety	A	B	21	22	Clean soil controls	21 R	A	B	23	A	B
Wm Copland	18	17	2	0	20*	3	0	0	0	18	13
Wm Pitt	20	19	20	0	19**	14	0	0	2	18	16

* Average of four boxes. ** Average of two boxes.

The strain (no. 24) from tomato was not so disastrous in its effects on tulips as the strains from tulip, or even the one from aster (Pl. XXVI, fig. 3). The intensification of parasitism with continued growth on the same host is no new phenomenon, and, as shown in Table III, the virulent strain 22 from tulip did not give a single flower from 120 bulbs: indeed, in the first season not a single shoot of Wm Copland appeared from twenty planted bulbs. Table III shows that *P. cryptogea* is as vigorous a parasite of forced tulips as *P. erythroseptica*. Both species have frequently been found, either independently or together, causing serious losses on nurseries every year since the work was begun, but the field of commercial growing has hardly been covered completely enough to justify

any opinion as to which species is the more commonly implicated. The greatest difference noted between the two species was the relative virulence to varieties, and this was not sufficient to be of practical importance.

Caution is necessary in diagnosing tulip diseases in the absence of pure cultures. *P. erythroseptica* may occasionally produce the proliferating sporangia shown by Pethybridge & Lafferty (1919) to be a characteristic feature of *P. cryptogea*. The presence of an odd proliferating sporangium in water cultures is not sufficient, therefore, to indicate definitely *P. cryptogea*, although the comparative frequency may be significant to an experienced observer. Roots alone rarely produce oogonia in water cultures but, occasionally, oogonia of *P. erythroseptica* may be seen from fleshy material after 24 hr. and, if the sterile water is changed to keep the cultures comparatively free from bacteria, they are usually abundant by the end of a week. This is in marked contrast to *P. cryptogea*, since it is very rare for this species to produce oogonia in water cultures, or in young pure cultures on agars.

Soil contamination as a factor in the occurrence of the disease on nurseries

The high proportion of flowers, as well as the clean roots, obtained in the controls in the experiments already described indicate that no disease was carried by those bulbs. On the other hand, the fact that both species of *Phytophthora* concerned form oospores, together with the ease with which the disease was produced when pure cultures were mixed with the soil, strongly suggests the latter as the chief means by which the disease is carried over. Nevertheless, especially in view of the disinclination of growers to accept this view, further experimental results may be quoted to emphasize the point.

In the early stages of the investigation, in May 1930, the trash and new bulblets were riddled out of a box of infected Bartigon plants brought back from Hampshire, and the soil was kept through the summer in a box exposed in the garden. In the autumn, thirty-six bulbs of Wm Pitt were planted in unsterilized garden soil which had not carried bulbs of any kind for a considerable number of years, but some of the old Hampshire soil was mixed with it just below the planted bulbs. On taking the boxes into the glasshouse there were only eight shoots, and only two of the plants ultimately produced poor flowers. At the same time numerous pure cultures of *P. erythroseptica* were obtained from the plants that had failed. On the other hand, thirty-six bulbs from the same con-

signment planted in sterilized garden soil gave a complete stand of shoots and thirty-four good flowers were obtained. That the infection came from the old Hampshire soil was clear from the fact that forty-two Bartigon bulbs in the unsterilized garden soil gave a perfect stand of shoots when taken out of the ash bed. They were forced under different conditions and only twenty-two flowers were finally cut but, in sterilized soil under the same bad forcing conditions, only eighteen Bartigon flowers were cut from an equivalent number of bulbs.

In later experiments the bulblets and trash were sometimes riddled out soon after the parent bulbs had been forced in soil contaminated with cultures, but in any event before any rooting of the bulblets had taken place, and at least a month before the new bulbs were planted. Although the boxes were given an occasional light watering during the summer, they were kept separated (to prevent infection spreading from box to box) and the soil became dry from time to time—certainly drier than it would get with the boxes stacked, or soil thrown in a heap, as under commercial conditions. The soil in each series was thoroughly mixed and filled into new, sterile boxes, before the fresh set of experiments was begun.

The first trials with soil that had previously borne an experimental diseased crop were carried out in 1931–2, but the plants met with such unusually poor growing conditions that it would be confusing to quote the actual flowering results. Only *P. erythroseptica* was concerned, and for reasons which were not clear there was a very poor "carry over" when Wm Copland was the test host. Wm Pitt grown in other portions of the same contaminated soil, however, gave numerous failures definitely due to the fungus. The results obtained in subsequent years are given in Table IV. Figures are also given for comparable bulbs planted in the same, but sterilized, lots of soil.

During 1933–4 *P. cryptogea* failed to give definite evidence of "carry over" when Wm Pitt was grown, although the crop of Wm Copland was reduced to 10%. In this instance the comparative failure of the parasite seems to be more definitely connected with the apparently lower susceptibility of the variety to the particular species. On the whole, when Table IV is considered in conjunction with Tables I and III, and supported by observations on nurseries, etc., there is abundant evidence that both species may remain virulent in the soil from one season to the next. No attempt was made to find how many years the parasites could live in soil left either in a heap or used for other crops, since the important point for the grower is the source of infection. Once it is shown that the

Table IV. *Numbers of flowers produced when tulips were grown in soils that had, during the previous season, carried plants attacked by* P. erythroseptica *or* P. cryptogea, *compared with the crops in the same soils sterilized*

Contaminant, season and treatment	Number of bulbs planted		Number of flowers cut		Percentage number of flowers cut	
	Copland	Pitt	Copland	Pitt	Copland	Pitt
A. Soil contaminated with *P. erythroseptica*						
1932–3						
Untreated	120	107	76	20	63·3	18·7
Soil sterilized	180	179	179	177	99·5	98·9
1933–4						
Untreated	40	80	2	33	5·0	41·2
Soil sterilized	40	80	38	78	95·0	97·5
B. Soil contaminated with *P. cryptogea*						
1932–3						
Untreated	20	15	1	21	5·0	60·0
Soil sterilized	20	179	18	177	90·0	98·9
1933–4						
Untreated	80	40	8	39	10·0	97·5
Soil sterilized	80	40	80	39	100·0	97·5
Untreated*	60	20	57	20	95·0	100·0

* This untreated soil was originally contaminated with the weak strain 24 from tomato. Although not very virulent to tulips, one of two tomato plants placed in the box in which a few tulips failed became attacked by foot rot.

disease can carry over in soil it is easy to point to bad practices that help the parasites to persist. The grower who allows contamination to become serious in a large bulk of bulb soil does not usually take adequate steps to prevent accidents when the boxes containing diseased plants are "run out". Moreover, it is difficult to remove all the bulblets from soil that has been used for forcing bulbs, and the longevity of a fungus in the soil depends to a large extent on whether the soil is free from "ground keepers", and even from slightly susceptible hosts. The parasites may also be carried over in the clods of old soil left in the corners of boxes. It is of interest to note that, although the disease occurs in outdoor plantings when the soil is deliberately contaminated, in bulb growing practice, where it is comparatively simple to arrange for rotation of the crop, no occurrence of the disease has been reported.

The mode of infection of bulbs planted in contaminated soil

To obtain more definite information about the way in which plants are attacked, bulbs were planted in soil heavily contaminated with *P. erythroseptica* and *P. cryptogea*, and were treated as for forcing. At intervals some of them were carefully lifted, washed and examined both macroscopically and in water cultures, etc., in the laboratory. The course

of events may be followed best from a brief description of one set of experiments using *P. erythroseptica.*

Wm Pitt bulbs were planted both in clean soil "controls" and in the contaminated soil on 17 September 1931. By 5 October, i.e. 18 days after boxing, only one out of eight bulbs examined showed any root production, while after 25 days half of the bulbs, whether in clean or contaminated soil, showed little or no root development. The optimum temperature for these *Phytophthora* spp. is 25–27·5° C., and as the soil is beginning to cool off rapidly at this time of the year it is probable that the irregularity in root production determines to some extent the degree of infection of the tulips, and accounts for the contrasts between badly diseased, and almost healthy plants that are always so perplexing to the grower: a perplexity that is increased owing to the fact that no heap of soil is ever uniformly contaminated. Some of the bulbs showed dead root tips while, in the worst affected, a number of roots were killed back to their point of attachment to the basal plate. From these roots sporangia of the parasite were obtained. A week later, 32 days after planting, there was still a great variation in the amount of rooting even in the controls. Of twelve plants from the contaminated soil, one with practically no roots, three with very short roots, and one with moderate roots showed no signs of infection; on the other hand, four of the others showed some of their roots infected and discoloured, while the remaining two not only had many roots discoloured, but the attack had also progressed into the basal plate. Indeed, in one of these bulbs the infection and discoloration had passed through the basal plate to the base of the flowering stem. Sporangia, pure cultures, and later oogonia were obtained from these six affected plants. It is not certain whether the fungus enters only through the root tips or whether it can directly infect the older portions of roots. Where, on a casual examination, the older portions only appeared to be attacked it was usually possible to trace mycelium in the tissues between the dead area and the root tip. Nevertheless, it is probable that the *Phytophthora* can gain access through wounded tissue, although the presence of many unattacked roots on a diseased bulb indicates that the uninjured, older portions of roots are more resistant. By 39 days (26 October) after planting, five of ten bulbs examined showed obvious rotting within the bulb, and the basal plate of six of them was definitely infected, and yielded abundant sporangia and oogonia in water cultures.

After 52 days (9 November) the attack on the flowering shoot had proceeded far enough to be readily seen in photographs (Pl. XXVI, fig. 4). Growth of the shoots had been arrested within the bulbs whereas, in clean soil, shoots projected $\frac{1}{2}$–1 in. above the noses of the bulbs. Abundant oogonia were again produced in isolations from the affected plants. Even 66 days after boxing the diseased plants had a reasonably good root system—a feature very frequently noted—although closer comparison showed a considerable difference in the amount of root between the infected plants and the controls. Further, many of the roots which had not rotted on the diseased bulbs were slightly discoloured and water soaked. Nevertheless, three out of ten plants from the contaminated soil were still apparently healthy inside. When two boxes of the same variety in equally contaminated soil were taken into the glasshouse each showed five reasonably good shoots from twenty bulbs, but all ten of these shoots failed subsequently, only one of them developing as far as producing a dried up flower bud; whereas, in clean soil, thirty-eight bulbs gave thirty-six flowers.

The experiment was repeated during the following season with both Wm Copland and Wm Pitt, and with *P. cryptogea* also as a soil contaminant. The results were similar, and no essential difference could be observed between the mode of infection of the two species. Infection has never been found to occur through the shoot above the nose of the bulb, and the experiments confirmed the method of infection deduced from examination of diseased plants from nurseries.

Symptoms after transfer to the glasshouse

A brief description may be given of the symptoms after transfer to the glasshouse. With a very virulent strain, or when the soil is heavily contaminated, there may be no healthy shoots in a box when the outdoor covering is removed, but frequently a comparatively full stand develops, and there is nothing abnormal to suggest that the money spent on fuel and housing is likely to be wasted. During the 4–6 weeks of the forcing period some of the shoots remain almost stationary, but gradually become more unhealthy in appearance until they can be twisted readily out of the bulbs, and show shrivelling and rotting of the tissues at the base. Few or many of the plants will often appear to be unfolding their leaves normally, and it is not until the first flower buds are showing colour that the tips of many of the leaves may fade, frequently with the development of red pigment, and the grower realizes that the plants are in a typically "shanked" condition. When the plants are lifted many of the roots remaining are yellowish. It is common on nurseries for some of the root stumps to be strongly discoloured, but this more intense browning seems to be due to the action of secondary organisms. In the same way there may be some rotting of the bulb scales, but this is not typical of *Phytophthora* attack *per se*, although the primary parasite often destroys completely the developing new bulb. This point, and the contrast between the rapid and complete rotting of some tissues at certain times, and the apparent barriers to progress in some of the older tissues, will be mentioned later in dealing with the symptoms in outdoor plantings. It should be stated here, however, that under experimental conditions a striking feature is the solidity and freedom from rotting of the scales of the parent bulb, even after many weeks in the glasshouse, and despite the complete rotting of the flowering shoot within the bulb. Indeed, by chance a box of bulbs that is so badly diseased that no flowers are produced may yield a larger crop of new bulblets than one which has all the flowers and foliage cut for market.

SPREAD OF THE DISEASE FROM ARTIFICIALLY INFECTED BULBS

Although a physiological disease, "topple", has symptoms resembling the tulip blossom blight attributed to *P. Cactorum* (Stevens & Plunkett, 1925), the latter has not been observed in this country, and there has been no indication that *P. erythroseptica* and *P. cryptogea* can infect the green stem of the tulip, or that aerial spread of infection can occur.

In the autumn of 1930, Wm Pitt, Wm Copland and Bartigon bulbs were planted in clean soil, and six widely separated bulbs in each box were inoculated with *P. erythroseptica* through deep wounds towards the centre of the basal plate. In some bulbs the wound was so situated, and the rot spread so rapidly, that no roots were formed, while in most bulbs the attack was serious enough to prevent the appearance of any shoot. The results may be illustrated best by giving some details of the Wm Pitt bulbs, the variety showing the most serious effects. Thirty sound bulbs were planted so that twenty-six of them were adjacent to one or other of the six inoculated bulbs. All had produced good shoots by the time they were taken into the glasshouse, while only one side shoot marked the positions of the six inoculated tulips. It was clear from isolations that the failures were due to *P. erythroseptica*, and that the disease which developed subsequently in some of the adjacent plants was also due to this fungus. The amount of spread during the forcing season was, however, comparatively limited. None of the plants showed the very poor growth so characteristic of bad outbreaks of the disease; only three "shanked", and three affected at the base of the stem gave poor flowers. Two other plants failed to give a satisfactory bloom, but there were similar failures due to factors other than the parasite in bulbs of the same variety in clean soil. In spite of the presence of six active centres of the disease, thirty bulbs gave twenty-two flowers of reasonable quality as compared with thirty-four from the larger number of thirty-six bulbs in clean soil.

During the following season two or four bulbs in the middle of boxes of Wm Copland and Wm Pitt in clean soil were inoculated with either *P. erythroseptica* or *P. cryptogea*. The inoculated bulbs usually failed completely, and though the pathogen was reisolated from an occasional neighbour the number affected was usually small. The worst instance of spread was again with the variety Wm Pitt in forty-four bulbs planted around four bulbs which failed completely after being inoculated with *P. erythroseptica*; forty of the forty-four uninoculated bulbs gave flowers, though four of them were of poor quality.

From 1931 to 1934 other experiments were carried out in which "mummified" bulbs, that had been rotted by the *Phytophthora* spp. in outdoor plantings, were placed at the centre or corners of boxes of bulbs in clean soil. On several occasions sufficient infection of the roots of neighbouring plants was obtained to show that the fungus was active in the "mummified" bulbs but, even in the worst box, only one neighbour "shanked".

Evidence is also available from other sources, since it has been noticed that, in experiments, one plant may gradually fail from the day it is taken indoors, though all its neighbours may produce good blooms.

Experimental production of the disease in outdoor tulips

All the evidence of the disease in outdoor plantings has been obtained from the experimental plots. The disease may have been overlooked out of doors because its symptoms are less severe, and also because they can so readily be confused with those of plants suffering from a basal plate damaged before planting, from careless hoeing in the early stages of growth, or from the attacks of mice or slugs. The lower temperatures prevailing during the growing season for garden tulips probably minimize the effects of the disease, while rotation of soil can be practised more readily than by many large forcers.

Sound bulbs were planted at the end of October in soil which had not carried bulbs for many years, and on some of the plots the soil was contaminated by putting several pieces of agar from pure cultures of either *P. erythroseptica* or *P. cryptogea* at the bottom of the dibble hole. In 1930–1 every bulb produced a shoot above the surface of the ground despite the fact that the depth of planting was several inches more than for bulbs in boxes. Before flowering, however, about one in eight of the Wm Pitt plants contaminated with *P. erythroseptica* showed dwarfing of the foliage to varying degrees, accompanied by conspicuous reddening (Pl. XXVII, fig. 1). In 1931–2, either through using larger quantities of inoculum, or because the winter was milder, there were numerous failures to appear above ground by early March in the Wm Pitt bulbs where *P. erythroseptica* was the contaminant, and as many as thirty-seven out of eighty-two bulbs failed thus. In no season, however, was there any appreciable number of "gaps" with Wm Copland bulbs contaminated with either fungus, or with Wm Pitt bulbs in soil contaminated with *P. cryptogea*. A cold spring followed and little change was seen until the foliage began to redden towards the end of April, when some of the plants were markedly dwarfed. The most serious case was that of the Wm Pitt

plants infected with *P. erythroseptica* already mentioned: in one row of forty-one bulbs there were twenty-one complete failures, three failed before flowering time, seven gave poor quality blooms, and only ten flowered satisfactorily.

Allowing for the lower temperature conditions, there is no essential difference between the progress of the disease in the open and indoors. Infection begins in the roots and passes through the basal plate to the flowering stem (Pl. XXVII, fig. 2), producing all stages of failure from a complete "miss" to a plant which produces a good flower, but later gives diseased new bulbs. The parasites were repeatedly reisolated, and the isolates used to reproduce the disease. The new bulbs sometimes failed early in the growing season, as already mentioned, but commonly large new bulbs were produced some, but not all, of which rotted completely by the time of digging, or occasionally after a period in storage, but before the new bulbs were finally cleaned. These infected bulbs, which gradually dried out to a "mummified" condition, were produced in both varieties irrespective of the parasite involved, and they were used in experiments described above.

In certain tissues the progress of the rot is sometimes comparatively rapid, but there are barriers to the extension of the rotting that are not completely understood. It is a common experience that the new bulb is sound although the flowering stem failed, or that the chief new bulb, and some of the offsets are completely rotten, while other adventitious bulbs are quite healthy, and remain healthy even if left in the old trash in store for a time. McKenny Hughes (1934, p. 118) refers to "the time at which the new bulbs and offsets become cut off by dead tissue from the still growing and flowering stem which perishes with the season of flowering", and it is probably this "dead tissue" which forms a barrier to the mycelium of the *Phytophthora*, in much the same way as it was thought to be a barrier to virus infection.

VARIETAL SUSCEPTIBILITY

Many more bulbs of Wm Copland are forced than any of the other varieties. Further, it is on the nurseries forcing large numbers of the popular varieties that the disease is more likely to be perpetuated. These factors, coupled with the variation in the intensity of contamination from place to place, and time to time, make it difficult to assess exactly the available evidence on the relative susceptibility of varieties. Amongst the Darwin varieties, Wm Pitt and Wm Copland, which have been used chiefly in these experiments, are highly susceptible. Rose Copland and

Bartigon are also attacked freely and Pride of Haarlem and Madam Krelage to a less extent, but Clara Butt appears to be relatively resistant. Among the Early Single varieties the disease has been seen in Vermilion Brilliant, White Swan and White Hawk, and the Double Early variety Tea Rose is also susceptible. In contaminated plots out of doors the May-flowering Cottage Tulip variety Inglescombe Yellow seems to be very resistant.

If there is any difference between the pathogenicity of the two species of *Phytophthora*, it is probably more apparent in the varietal reaction than in other features of the disease. The tentative opinion of the writer is that Wm Pitt is less susceptible to *P. cryptogea* than to *P. erythroseptica*, while, although the evidence is not so consistent, it is possible that the reverse holds with Wm Copland.

Possibility of transmission with the bulb

When a bad outbreak occurs it is, perhaps, natural for the grower to attribute it to the purchase of infected bulbs, which are usually imported. Nevertheless, no satisfactory evidence has been obtained to indicate that the bulb is ever responsible for a bad attack. Repeated appeals have been made to growers to send suspicious specimens culled from imported bulbs. Apart from occasional specimens attacked by *Penicillium*, and others damaged by exposure to hot sun at lifting time, or by some other circumstance, the only diseased bulbs that have been received proved to be infected with *Pythium* (Moore & Buddin, 1937). Growers familiar only with the Shanking phase conclude that the bulbs must be faulty when they find occasional "misses". These investigations have proved, on the contrary, that such complete failures may occur when the healthiest bulbs are planted, if the attack originates from contaminated soil. Growers are also prone to conclude that it is only the batch of plants from one particular source that is affected, whereas careful inspection usually reveals mild attacks in plants from other consignments.

In experimental plots a large proportion of the affected new bulbs are completely rotten before the crop is lifted, while those which may rot subsequently are picked out during the cleaning and sorting operations. The action of the two species of *Phytophthora* is very similar to that of one of them, viz. *P. erythroseptica*, when it produces Pink Rot of the potato (Cairns & Muskett, 1933; Pethybridge, 1913). Cairns & Muskett (1933) stated that tubers, scarcely to be regarded as so free from soil and the possibility of attached oospores as tulip bulbs, will, if affected, "have become mummified by planting time and obviously unfit for use as

'seed'". Each tulip bulb is handled as much, and by equally intelligent labour, as a potato "set", and it is unlikely that affected bulbs would be used. Further, if bulbs bearing oospores of the fungi should be planted, it is clear from the experiments on the spread of the disease from an inoculated bulb that the effect is hardly likely to approach in seriousness the bad attacks frequently seen. One grower stated in 1930 that "in every case in the centre of the affected area of tulips" (forced in boxes) "there was one dead rotten bulb, and infection from this appears to have radiated to the extent of 8–10 in., killing, or partly destroying, the bulbs within that area. In short, one bulb which was dead when planted (*sic!*) and never showed any signs of life had killed about 50 others round it." The improbability of this statement is evident from the experiments recorded in this paper.

Experiments, carried out with entirely negative results on the point at issue, may be described briefly. In 1934 a grower submitted a sample of suspected bulbs. The thirty bulbs in clean soil produced twenty-nine good flowers; the remaining bloom was imperfect, but the plant was healthy. On the other hand, 5–10% of the plants were badly affected with *P. cryptogea* when the main batch of bulbs was planted in the grower's own soil. Previous experiments included growing the new bulblets derived from experimental and commercial diseased plants. No signs of the disease were seen, although, under identical conditions, similar bulblets grown in soil contaminated with pure cultures showed abundance of the disease. Bulbs of Wm Copland, Wm Pitt and Bartigon have been raised repeatedly in contaminated soil, the infectiveness of which has been demonstrated by some of the crop being diseased. Such bulbs have been forced or grown out of doors without obtaining any evidence that the parasites might be carried on bulbs subjected to the customary cleaning and grading operations.

Positive evidence may yet be obtained that the two species are occasionally carried on the cleaned, and much handled, bulbs, but there seems little doubt that soil contamination is responsible for severe outbreaks.

CONTROL OF THE DISEASE

The possibility of contaminated water supply

With diseases caused by certain species of *Phytophthora* the possibility of a contaminated water supply must always be considered. On a nursery where one of the earliest severe attacks occurred the water was examined at the time of the outbreak by the method of Bewley &

Buddin (1921) with entirely negative results. On one nursery where there had been repeated attacks the water supply may have been contaminated, since it was drawn from an open stream, and from a point *below* the spot on the waterside where the soil and refuse from previous diseased tulips had been dumped, but the water was only one of the probable sources of infection even on that particular nursery.

Common pitfalls in connexion with soil

It has already been pointed out that soil brought in from a distance is not necessarily free from parasites such as *P. erythroseptica* and *P. cryptogea*. Contamination of "new" soil with the latter is known to cause outbreaks of "damping off" etc. in tomato and other plants, while the use of soil that has previously grown a crop of tomatoes is an obvious way in which this disease may be initiated in tulips.

The advice commonly given that tulips "should follow potatoes, or some crop that has been well manured" (Hall, 1929, p. 196) also needs to be accepted with caution. Pink rot of potatoes is not a common disease in this country, but it does occur from time to time, and may easily be overlooked in the field, although the warmer conditions of the forcing house would probably bring it into greater prominence if the fungus were introduced in the soil. Cairns & Muskett (1933) found that satisfactory potato crops can be grown in soil contaminated with the pink rot organism, "provided that strongly sprouted tubers are planted, and that soil drainage is sufficiently good to prevent the land becoming unduly wet in times of heavy rainfall". How far similar reasoning may apply to the tulip disease is not known, but there seems little doubt that the heavy soil, careless watering and poor drainage of the boxes provided by some growers may aggravate the disease when the soil is contaminated. Further, the danger of contaminating imported, clean soil must be emphasized.

Partial sterilization of soil

(i) *Use of formaldehyde.* As soon as it was realized that infection could come from the soil, and a supply of suitable soil was available, the effect of soil treatment with formaldehyde was tested. Both species of *Phytophthora* were investigated, and the experiments were continued for more than one season. The strength usually recommended for soil sterilization (1 part of freshly purchased (38–40 %) formaldehyde solution to 49 parts of water) was used though, on account of the difficulties of treating small quantities in boxes, in amounts more liberal than the

standard dose. The soil was treated at least a fortnight before planting, and the boxes were covered with sacking steeped in the solution for two days after the actual treatment. Both ordinary commercial formaldehyde, and Steriform were employed, but no difference was noted in the effects, and the results are considered together (see Table V and Pl. XXVII, fig. 3). They were generally satisfactory, the treated soil giving an average of 97 % of good flowers, while only 30 % of marketable blooms was obtained from the same batches of soil left untreated. In 1936–7, when the treatment was, perhaps, carried out less carefully, only twenty-two flowers were obtained from twenty-five Wm Copland bulbs, and definite infection was present in the failures. This emphasizes the need for the greatest care in carrying out soil treatment, and in preventing re-contamination, but does not detract seriously from the value of formaldehyde in combating the *Phytophthora* disease. On the other hand, formaldehyde is not a panacea for tulip diseases in the forcing house, as was shown by work on Grey Bulb Rot (Buddin, 1937). Further, as Bewley (1935) states—"failure of chemical sterilizers is a constant feature of heavy soils, and there is little doubt that it is due to the impossibility of making intimate contact between the sterilizing agent and every particle of soil".

(ii) *Other chemicals.* It was suggested by Foister (1930) that the tulips should be watered with a solution of Cheshunt Compound as soon as the disease was observed. The investigation of the mode of infection made it clear, however, that such late soil treatment would be useless, though it did not rule out Cheshunt Compound for use at, or before, the time of planting.

In a preliminary experiment in 1931–2, soil contaminated with *P. erythroseptica* was watered with Cheshunt Compound several weeks before planting, or immediately after covering the bulbs with soil. The conditions for flowering were unsatisfactory, but there was clear evidence from the pure cultures isolated that some of the failures were due to *Phytophthora*. Further, when the soil was not treated until immediately before the boxes were covered with ashes, there was some evidence that the chemical itself affected the plants adversely under the rather static conditions for soil aeration and moisture during the rooting period. Such action is, perhaps, less likely under modern commercial conditions where the boxes are covered with straw.

The results obtained in subsequent years are summarized in Table V. The standard strength of Cheshunt Compound was used, but sometimes as much as twice the quantity ordinarily recommended was applied.

Table V. *Flowering in contaminated soil compared with that in the same soil when steam sterilized, or treated with formaldehyde (or Steriform) or Cheshunt Compound*

			Untreated control		Steam sterilized soil		Soil treated with formaldehyde or Steriform		Soil treated with Cheshunt Compound	
Season	Soil contaminant	Variety	No. of bulbs planted	No. of flowers cut	No. of bulbs planted	No. of flowers cut	No. of bulbs planted	No. of flowers cut	No. of bulbs planted	No. of flowers cut
1932–3										
	P. erythroseptica	Pitt	107	20	59	59	120	118	40	11
	P. erythroseptica	Copland	120	76	60	59	120	120	40	30
	P. cryptogea	Copland	20	1	—	—	20	18	—	—
1933–4										
	P. cryptogea	Copland	80	8	20	20	60	60	60	18
	P. erythroseptica	Copland	40	2	20	20	20	18	20	8
	P. erythroseptica	Pitt	80	33	20	20	60	58	60	41
1936–7										
	P. erythroseptica and *P. cryptogea*	Copland	50	12*	25	25	25	22	—	—
Totals			497†	152†	204	203	425	414	220	108
Percentage flowering			30·6†		99·5		97·4		49·1	

* A dozen flowers of very poor quality were produced in addition in this batch.

† The totals and the percentage flowering in the actual controls for each treatment are slightly different, but the variation in the percentage flowering is only from 30·6 to 32·5.

Table V shows that, although the Cheshunt Compound had a slight fungicidal effect, yet less than one half of the bulbs bloomed satisfactorily. The failures being due to the parasites, Cheshunt Compound cannot, therefore, be recommended even if the treatment be carried out before planting.

Other chemicals were not tried extensively. One proprietary article was tested on duplicate boxes of contaminated soil, but the control obtained was not as good as with formaldehyde, and the cost would be greater.

(iii) *Use of steam.* The value of steam sterilization is clearly shown in the results summarized in Table V. The contaminated soil averaged little more than 30% of flowers, while the same soil, subjected to routine steam sterilization, gave 99·5% of marketable blooms (Pl. XXVII, fig. 4). Although steam is of almost universal value for the eradication of disease when the value of the crop justifies its cost, growers are sometimes sceptical of the type of growth which may result. No statistical data can be presented, but it may be said that in no instance did steaming the soil—carried out a fortnight or so before planting—have any adverse effect on growth: on the contrary, the largest plants and the finest blooms were invariably produced.

SUMMARY

Phytophthora erythroseptica and *P. cryptogea* have been isolated on numerous occasions from diseased tulip plants on commercial nurseries, and the isolates, both in pure culture and in their effect on the host, have been compared with authentic cultures of these fungi. Both species have been proved to be, either separately or in conjunction, the cause of a serious root rot, shoot rot and shanking of forced tulips.

Infection, which takes place from contaminated soil through the roots, begins as soon as the first roots are produced from the basal plate of the bulb. The parasite passes directly through the basal plate to the base of the flowering stem, leaving the old bulb scales practically unattacked. According to the time, or rapidity, of infection an affected bulb may produce no visible shoot, or it may give a shoot bearing a flower bud that remains blind because the flower stem is rotted at the base (shanking). Sometimes a bulb with some of its roots attacked may produce a marketable flower of poor quality.

A very similar disease has been induced in outdoor tulips by contaminating the soil.

The disease, which may result in the total loss of a forced crop, has not yet been reported outside Great Britain, where it seems to be widely distributed.

There is no evidence that the disease is carried with the planted bulb in ordinary commercial practice, and the rate of spread in the glasshouse from one or more inoculated bulbs planted in the midst of healthy ones is comparatively slow.

The susceptibility of varieties of tulips to the two species of *Phytophthora* is briefly discussed.

A contaminated water supply is a possibility, but appears to be relatively unimportant.

Partial sterilization of the contaminated soil by steam, or formaldehyde, combined with hygiene, is an effective method of controlling the disease, but soil treatment with Cheshunt Compound is unsatisfactory.

Certain strains of *Phytophthora cryptogea* var. *Richardiae* and *P. parasitica* were tested, but appear to be of little, or no, consequence as parasites of forced tulips.

A large proportion of the success of the present investigations has been due to the stimulus, and ever-ready helpfulness, of Dr Geo. H. Pethybridge and Mr W. C. Moore. The credit for identifying the fungi concerned lies almost entirely with Mr S. F. Ashby. The writer also wishes to express his thanks to his Laboratory Attendant, Mr A. Sherval, for taking the photographs.

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Fig. 1.

Fig. 3.

Fig. 4.

Fig. 2.

BUDDIN.—Root Rot, Shoot Rot and Shanking of Tulip caused by *Phytophthora cryptogea* Pethybr. & Laff. and *P. erythroseptica* Pethybr. (pp. 705–729)

Fig. 1.

Fig. 3.

Fig. 2.

Fig. 4.

BUDDIN.—Root Rot, Shoot Rot and Shanking of Tulip caused by *Phytophthora cryptogea* Pethybr. & Laff. and *P. erythroseptica* Pethybr. (pp. 705–729).

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EXPLANATION OF PLATES XXVI AND XXVII

PLATE XXVI

Fig. 1. A "shanked" Wm Copland tulip with shrivelled flower bud and poorly developed, fading foliage consequent on attack on the roots by *Phytophthora cryptogea*; the bulb has been cut to show that the rotting has proceeded through the basal plate and affected the base of the flowering stem which is slightly discoloured. The original specimens for which the name "shanking" was suggested had probably been attacked earlier, or by a more virulent strain, and showed more shrivelling of the base of the flowering stem than the plant illustrated.

Fig. 2. Wm Pitt tulips in soil contaminated with *P. erythroseptica* (*a*, strain 1 from tulip; *c*, strain 6 from potato) contrasted with plants from the same number of bulbs in similar, but clean soil (*b*). Photographed 22 February 1932.

Fig. 3. Wm Copland tulips (*a*) in soil contaminated with *P. cryptogea* (strain 21 from aster) compared with plants (*b*) grown under the same conditions in clean soil. Photographed 26 January 1932.

Fig. 4. Bulb of variety Wm Pitt (*a*) cut 52 days after planting in soil contaminated with *P. erythroseptica*; the roots and basal plate are attacked, as also the base of the flowering stem. The bud which should eventually form the new bulb is completely rotten. Bulb from clean soil (*b*) for comparison. Photographed 9 November 1931.

PLATE XXVII

Fig. 1. Wm Pitt tulips in outdoor plot in soil contaminated with *P. erythroseptica*. Note the stunting of the affected plants, much of the foliage of which had reddened. Photographed 9 May 1931.

Fig. 2. Wm Pitt tulip bulb planted out of doors on 30 October 1931 in soil contaminated with *P. erythroseptica*. The rot has progressed from the roots up the flowering shoot, but the old bulb scales are not attacked. Photographed 21 March 1932.

Fig. 3. Wm Copland tulips in soil contaminated with *P. cryptogea* that had produced a diseased crop during the preceding season. *a*, soil treated with formaldehyde several weeks before the planting date; *b*, soil left untreated. Photographed 31 January 1933.

Fig. 4. Wm Copland tulips in soil from a nursery contaminated with both *P. erythroseptica* and *P. cryptogea*. (*a*) soil steam sterilized; (*b*) soil left untreated. Photographed 9 January 1937.

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INVESTIGATIONS ON THE RUST OF ROSES *PHRAGMIDIUM MUCRONATUM* FR.

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Introduction

The rust of roses caused by *Phragmidium mucronatum* has of recent years become increasingly prevalent, especially on stocks grown for grafting. Where a severe attack occurs on such plants, retardation of growth due to premature defoliation and to the death of branches infected by the caeoma stage may result in heavy financial loss to the grower. It has also been suggested that roses on an infected nursery may contract the disease from the infected briars and be responsible for its introduction into gardens hitherto free from it.

At the request of the National Rose Society, an investigation of this disease was commenced in 1933. The work has been concerned mainly with two problems, the possibility of cross-infection from the briar to the rose, and overwintering. Certain other questions have also received attention.

Phragmidium mucronatum produces all spore forms. The aecidiospore stage is found mainly on buds and stems where it often forms large caeomata. It may also occur on leaves. It apparently plays a large part in the overwintering of the fungus. On the leaves the uredospore stage is most common and is responsible for the spread of the rust during the summer. The teleutospores arise in the uredosori in the autumn and

uredospores and teleutospores are commonly found together in the same sorus. In the late autumn teleutospores predominate and may be the only spore form present.

Both aecidiospores and uredospores have been found to infect suitable hosts readily. Eriksson (1924) reports that aecidiospores from overwintered mycelium on *Rosa rubrifolia* failed to infect, while aecidiospores from fresh infections did so. This observation has not been confirmed in the present investigation. In one case a plant which showed caeomata in 1935 produced a further zone on the same branch in 1936 below that of the previous year, although it had not been exposed to fresh infection. Spores from this second caeoma caused infection. The mycelium had, in this case, been at least two years in the plant.

Conditions for the natural spread of the fungus would appear to be unfavourable at Cheshunt. Infected plants of *Rosa canina* were planted on the nursery in 1933. A certain amount of spreading to neighbouring plants of *R. laxa* occurred in 1934 but the disease gradually died out, although, in order to avoid possible loss of caeomata, the plants were not pruned. Attempts to infect roses and *R. laxa* in a large experimental glasshouse were also unsuccessful. In one experiment, plants placed on the bench immediately after inoculation became infected, but usually it was found necessary to place the plants in a moist chamber to secure infection.

Experiments were carried out to determine the minimum time of exposure to moist conditions which would result in infection. Using aecidiospores from Kokulensky's *canina* as inoculum, plants of *R. laxa* kept in the moist chamber overnight became infected. A similar result was obtained with uredospores of the same strain. The actual period in the moist chamber was 13–14 hr. Infection would probably follow much shorter exposures to moist conditions since, in one experiment, a plant became infected after only 4 hr. in the moist chamber. This experiment was, however, carried out by day when it proved impossible to maintain a saturated atmosphere in the moist chamber for more than a short period. The results were therefore inconclusive.

The failure of the fungus to infect save in the moist chamber had the incidental advantage that cross-infections between strains from different sources were very rare, only two cases occurring among over 500 plants inoculated.

Strains of the fungus

(*a*) *Infection experiments*

Many rose growers believe that rust on the cultivated rose has become more widespread since the use of *R. laxa* as a stock has become more common. It has been suggested that the stock may influence the reaction of the rose to the rust, but no evidence in favour of this hypothesis has been obtained in the present investigations. Alternatively, it has been maintained that a new race of *P. mucronatum* has been introduced on *R. laxa*. This race is said to be different from the native form on *R. canina* and capable of infecting the rose.

The first evidence that there might be physiological forms of *P. mucronatum* was obtained in 1934, when repeated attempts to infect *R. laxa* with rust from the cultivated rose failed. Roses inoculated at the same time were freely attacked. It was decided to carry out comparative experiments with rust from different sources, using as test plants the cultivated rose, *R. laxa* and several strains of *R. canina*. By the courtesy of Mr Courtney Page pot plants of the following were obtained.

(*a*) Deegen's *canina*.
(*b*) Kokulensky's *canina*.
(*c*) Schmidt's Special.
(*d*) *R. canina* (unnamed strain).
(*e*) *R. laxa*.
(*f*) Rose Margaret McGredy on seedling briar.
(*g*) Rose Margaret McGredy on *R. laxa*.

During 1935, a series of inoculation experiments was carried out using the following strains of the rust:

A. Uredospores on leaves of *R. canina*.
B. Uredospores on leaves of *R. laxa*.
C. Uredospores on leaves of cultivated rose.
D. Uredospores on leaves of *R. rugosa*.
E. Aecidiospores on stems of *R. canina*.
F. Uredospores on leaves of *R. laxa* (a second collection).
G. Aecidiospores on leaves of cultivated rose.
H. Aecidiospores on buds and stems of Kokulensky's *canina*.
K. Aecidiospores on buds and stems of Deegen's *canina*.

Strain D from leaves of *R. rugosa*, unfortunately died out before the full series of test plants was available, and only its reactions on the rose and *R. laxa* were tested. Several of the strains (those from

briars) ceased production of uredospores at the end of the year and it was impossible to keep them alive over the winter. Those on the rose, however, survived and it was possible to reisolate strains E, H and K from caeomata on their respective hosts in the following spring.

In each experiment all the test plants were inoculated at the same time. Spores were transferred to the healthy leaf by means of a fine needle. The topmost four or five fully developed leaves on a growing shoot were inoculated, thus eliminating the effect of the age of the leaf on infection. The inoculated shoot was marked and the remainder of the plant served as control. In order to ensure adequate exposure to moist conditions the inoculated plants were kept in the moist chamber for 72 hr. and inoculations were made in the evening so that the falling temperature would cause almost immediate saturation of the atmosphere and avoid desiccation of the spores. The plants were subsequently placed on the bench in the glasshouse, each strain being isolated by curtains of cheesecloth. In most cases each experiment was repeated several times.

The reactions of each strain are given in detail below. The reactions of the two roses were identical and they are not referred to separately although they were always tested.

A. *Uredospores on leaves of* R. canina.

This strain never produced very abundant infection even on its original host out of doors. In experimental transfers it caused slight infection with very small sori on *R. laxa*, Deegen's *canina* and the unnamed *R. canina*. The sori were often paler in colour than those of other strains, probably owing to paucity of spore production. It did not infect the cultivated rose, Kokulensky's *canina* and Schmidt's Special. The feebleness of its growth both in the glasshouse and on *R. canina* out of doors prevented more than one complete test.

B. *Uredospores on leaves of* R. laxa.

Yellow marks were produced on the upper surface of the leaves of the rose in one experiment out of four. It would appear that penetration took place to some extent, but there was no definite infection. Positive infection with spore production occurred on all the other hosts. The sori on all except Schmidt's Special were about 0·5 mm. in diameter and orange yellow in colour. On Schmidt's Special the sori were very small but numerous and bright yellow in colour. On *R. laxa*, Deegen's *canina* and Kokulensky's *canina* yellow areas appeared on the upper surface of the leaf corresponding in position to the sori on the lower.

C. *Uredospores on leaves of the cultivated rose.*

The cultivated rose was readily infected by this strain. On Schmidt's Special necrotic areas with a few very small sori were produced in one experiment; in a second, necrotic areas only appeared; while, in a third, no effect was observed. The other briars were not infected in three trials, although roses inoculated at the same time were attacked. In one experiment, a plant of Kokulensky's *canina* showed rust on both

noculated and control leaves. This rust could not be transferred back to the rose and was, doubtless, a chance infection by one of the briar strains.

D. *Uredospores on leaves of* R. rugosa.

As mentioned above, this strain, which was collected in the summer of 1933, died out before it could be tested on the full range of hosts. During 1933 and 1934, however, six attempts were made to infect *R. laxa* with it. All failed, although inoculations of the rose made at the same time were successful.

E. *Aecidiospores on stems of* R. canina.

The fungus derived from the caeomata on the *R. canina* plants at Cheshunt was more vigorous than that from the leaves of the same plants (strain A), although, doubtless, it was the same. Reddish brown marks were sometimes produced on rose leaves but no spores. Kokulensky's *canina* was not infected in five trials. Schmidt's Special was infected once out of four trials, Deegen's *canina* in five out of six, and the unnamed *R. canina* in three out of four. Only on Deegen's *canina* were the sori of normal size.

F. *Uredospores on leaves of* R. laxa.

In August 1935, leaves of *R. laxa* were received from Mr Courtney Page which were heavily infected by rust. The reactions of this strain differed markedly from those of strain B from the same host. On the rose, one very small sorus appeared on one plant in the first test, the second plant showing light green to yellow marks on the inoculated leaves. In a second trial no apparent effect was produced. On *R. laxa*, infection was very heavy when the strain was new and spore production was very abundant; there were yellow spots on the upper surface of the leaf. On Deegen's *canina* there were numerous small sori in two experiments and, on the unnamed *R. canina*, a few small sori in the first trial. Kokulensky's *canina* and Schmidt's Special were not infected. Unfortunately, the lateness of the season prevented more than two tests and the rust did not survive the following winter.

G. *Aecidiospores on leaves of cultivated rose.*

The reactions of this strain were identical with those of strain C, also from the rose. Small sori were again produced on Schmidt's Special, from which it was transferred successfully back to the rose.

Table I. *Host range of strains of* P. mucronatum

Strain	Source	Host: Rose	*R. laxa*	Kokulensky's *canina*	Deegen's *canina*	Schmidt's Special	*R. canina*
A	*R. canina*	−	+	−	+	−	+
B	*R. laxa*	?	+	+	+	+	+
C	Rose	+	−	−	−	+	−
D	*R. rugosa*	+	−				
E	*R. canina*	?	+	−	+	+	+
F	*R. laxa*	+	+	−	+	−	+
G	Rose	+	−	−	−	+	−
H	Kokulensky's *canina*	?	+	+	+	+	+
K	Deegen's *canina*	?	+	+	+	+	+

? = Necrotic lesions without spores.
+ = Infection with spore production.
− = No apparent effect.

H. *Aecidiospores from buds on Kokulensky's canina.*

This strain was identical in its reactions with strain B from *R. laxa*. It produced necrotic lesions on the rose but no spores.

K. *Aecidiospores from buds on Deegen's canina.*

The host range and reactions of this fungus were also identical with those of strain B.

The host range of these strains is summarized in Table I.

On the basis of these experiments the strains may be arranged in five groups:

(1) Strains A and E.
(2) Strains B, H and K.
(3) Strains F.
(4) Strains C and G.
(5) Strains D.

The fungi of group 1 resemble rather closely those of group 2, differing only in their inability to infect Kokulensky's *canina*. Strain D from *R. rugosa* is placed provisionally in a separate group for lack of information as to its host range. Probably it should be grouped with strains C and G from the rose. It was unfortunate that it proved impossible to obtain plants of *R. rugosa*, since tests on that host would have been of great interest.

The rose appears to be highly resistant to infection by the forms occurring on briars. In only one case (strain F) were spores produced and then only one very small sorus was formed. Allen (1927) reports a very similar situation with *Puccinia triticina* form 11 on the resistant Malakoff wheat. In that case the first invaded cells usually die immediately and the rust fails to gain a lodgement. Occasionally, however, the rust survives and forms haustoria. Feeble uredosori may even be produced. In the susceptible Little Club wheat on the other hand the host cells are not damaged until the infection is quite old and sporulation is normal (Allen, 1926). The infection of Schmidt's Special by the rose strains would seem to be on the same plane. It appears that this variety is somewhat less resistant to these strains than the other briars tested. The small size of the uredosori of the rusts of group 2 on this host suggests that it is also somewhat resistant to them. So far as these experiments go, therefore, it does not appear that cross-infection between briars of the *R. canina* and *R. laxa* groups and the rose takes place to any extent.

(*b*) *Morphological characters of the strains*

The physiological differences between the strains are, to some extent, correlated with morphological differences. Macroscopic examination did not reveal any significant variation among them when growing on suitable hosts. Measurement of the uredospores did not produce any useful information. It was found that the spores swelled in water and discharged their contents through the germ pores with a resultant secondary shrinkage. Measurements were therefore unreliable, since it was impossible to determine at what stage in this process a spore might be.

Measurements of the teleutospores, however, have given some interesting results. One hundred spores from each strain (except D) were measured. Only the actual body of the spore was considered, the pedicel and hyaline tip being neglected. The results are shown in Table II. Since the number of cells in the spore directly affects its length, the average length of each "cell class" is given separately. While no counts of fields were made, these figures give a fairly accurate idea of the proportion of each "cell class". The range of each class is shown in Table III. The width of the spores varies from 15 to 29 μ and is approximately the same in all strains.

Table II. *Average length of teleutospores in strains of* P. mucronatum

Measurements in microns

No. of cells	A	B	C	E	F	G	H	K
3	—	—	—	—	—	57·8 (1)	—	—
4	—	57·8 (2)	60·0 (1)	—	—	61·7 (8)	—	—
5	62·9 (3)	69·1 (8)	69·7 (19)	64·4 (1)	—	70·9 (30)	64·4 (3)	63·2 (7)
6	73·4 (19)	75·3 (22)	83·9 (48)	73·1 (12)	74·4 (2)	84·0 (49)	73·6 (23)	71·0 (19)
7	82·9 (51)	83·4 (46)	96·3 (32)	81·5 (53)	83·8 (25)	95·4 (12)	82·6 (51)	81·8 (48)
8	95·6 (26)	92·5 (20)	—	89·7 (34)	92·7 (41)	—	95·4 (21)	92·1 (23)
9	111·1 (1)	104·4 (3)	—	—	100·8 (30)	—	103·3 (2)	101·5 (3)
10	—	—	—	—	111·1 (2)	—	—	—

Numbers in brackets represent numbers of spores measured.

In strains A, B, E, H and K seven celled spores were predominant, in strains C and G from the rose six-celled, while strain F showed a large number of seven, eight and nine-celled spores, with eight cells the most numerous.

Table III. *Range in length of teleutospores in strains of* P. mucronatum

Measurements in microns

No. of cells	A	B	C	E	F	G	H	K
4	—	53·3–62·2	—	—	—	53·3–68·9	—	—
5	62·2–64·4	62·2–73·3	64·4–77·8	—	—	66·7–75·5	62·2–66·7	60·0–71·1
6	66·7–82·2	68·9–86·7	77·8–95·5	71·1–75·5	71·1–77·8	73·3–93·3	66·7–77·8	66·7–77·8
7	73·3–91·1	71·1–93·3	88·9–106·7	71·1–88·9	77·8–91·1	88·9–104·4	75·5–91·1	75·5–91·1
8	86·7–100·7	86·7–100·0	—	82·2–97·8	84·4–100·0	—	88·9–104·4	82·2–100·0
9	—	102·2–106·7	—	—	93·3–106·7	—	97·8–108·9	100·0–104·4
10	—	—	—	—	108·9–113·3	—	—	—

When the average length of spore in each class is considered, it is evident that with one or two exceptions the briar strains resemble each other closely while the spores of those from the rose are somewhat larger. In the case of spores with seven cells these differences were examined statistically. Twenty-five measurements were selected at random from each strain, except G, where the measurements were too few, and tested by the method outlined by Fisher & Wishart (1930). This examination showed that the average length of seven-celled spore in strain C was significantly different from those of corresponding spores in the briar strains, while the briar strains did not differ significantly among themselves. Grove (1913) figures teleutospores on Dog Rose and Burnet Rose which show similar variations in size. He regards them as belonging to the same species. Arthur (1929), however, considers the rusts on cultivated rose and certain wild roses to be distinct species. It is evident from the experiments described above that extensive collections of rust must be made and tested on a wide range of hosts before the exact status of these forms can be decided.

Overwintering

Phragmidium mucronatum is able to pass the winter in two ways, by means of teleutospores and as mycelium in the tissues of the host. Both of these stages appear to be of importance in the life history of the fungus.

(*a*) *Germination of teleutospores*

A period of dormancy is necessary for the proper ripening of the teleutospores of many rusts although the length of this period varies greatly in different species. Experiments have been carried out to determine its duration in *P. mucronatum* and how far it is affected by conditions of storage.

In conducting germination tests the following method was adopted. Shallow vulcanite rings were cemented on to glass slides rather towards one end. Spores were floated on distilled water contained in these rings and, also, placed dry on the slide to be moistened by the condensation water. The slides were placed in Petri dishes lined with moist blotting paper.

In the first experiment in 1933–4 leaves of *R. canina* bearing teleutospores were gathered at the end of August. It was decided to store them in glass jars with loose fitting covers and, since it had been found that fresh leaves so enclosed rapidly decayed, they were first air dried in the laboratory. One jar was kept in the laboratory while the other was placed out of doors in a glass-sided box which was open on one side. The top of this jar was protected from possible wetting by rain by a piece of oiled silk. The average weekly temperature and humidity from the commencement of germination tests were calculated from continuous records. In the laboratory the weekly average temperature varied from 55·2° F. in December 1933 to 70·8° F. in the last week of the experiment in July 1934. Over the whole period from October 1933 to July 1934 the temperature averaged 59·6° F. Outside, the lowest temperature occurred in November and December 1933, the lowest weekly average being 35·3° F. The highest, in July 1934, was 71·0° F. and the average over the whole period 49·0° F. Humidity records were not taken over the whole period in the laboratory, but averaged 48% during the period from October 1933 to March 1934. The corresponding figure outside was 77 and 74% over the whole period.

Spores were tested at fortnightly intervals from 30 October to 26 June 1934. Slides from each batch were placed under a range of conditions of lighting and temperature, in incubators at 25, 20 and 15° C., on the bench at room temperature, and in a glass-sided box on the bench which was controlled thermostatically at about 20° C. The slides were kept under observation for a long period, the majority until the end of the experiment on 18 July. In no case was any germination observed.

For the next season, a different method of storage was adopted. A supply of rose leaves bearing teleutospores was received from Mr

Courtney Page at the end of August 1934. They were divided into three lots and each lot was enclosed in a muslin bag. One bag was hung up in the laboratory, one in a glasshouse, and the third in the glass box out of doors. A further supply, received a little later, was placed in a desiccator over water and kept on the bench in the laboratory. The average conditions of temperature and humidity from October 1934 to September 1935 in these positions is shown below.

	Temperature ° F.			Humidity %		
	Weekly average		Whole period	Weekly average		Whole period
	Max.	Min.		Max.	Min.	
Laboratory	72·4	55·9	63·0	80·2	42·8	58·0
Glasshouse	76·0	56·7	66·1	87·6	71·2	79·0
Outside	72·5	40·6	53·8	83·2	60·2	71·9

Germination tests were commenced on 8 October 1934 and continued until 12 September 1935. The attempt in the previous year to investigate the effect of temperature and light as well as of conditions of storage on germination had led to an unwieldly accumulation of slides and, therefore, it was decided to make the tests at room temperature only. Tests were made at monthly intervals until germination was first observed at the beginning of May. They were then made at approximately weekly intervals for a short period.

Germination was observed in the four tests made on 8, 14, 20 and 29 May. In each case the spores which germinated had been stored outsid and were floating on water. No spores stored in the laboratory or in the glasshouse germinated, nor did any that had been placed dry on the slide. Of the very large number of spores used in each test only a very small proportion germinated, those tested on 14 May showing the largest number. The promycelia varied greatly in length and were colourless, except where a little of the yellow spore contents had been held up by the septa. The sporidia were bright yellow.

In 1936, teleutospores on rose exposed out-of-doors again showed germination during May.

From these experiments, it appears that the teleutospores of *P. mucronatum* require a period of rest and that exposure to winter conditions is necessary for their proper ripening. Exposure to cold alone does not appear sufficient, since the winter of 1933–4 was appreciably colder than the following one. In the winter of 1934–5 the spores, while protected from direct wetting by rain, were exposed to mist and fog, and it may be suggested that the preliminary drying and storage in jars in the previous year led to the death of the spores. It must, however, be

acknowledged in comparing the two years that the teleutospores from *R. canina* were of a different strain from those used in the following year and that the strain was not very vigorous, as shown above.

The small proportion of spores which germinated may, perhaps, be due to the conditions of the experiment. Possibly the leaves were picked before many of the spores were fully developed and only a small proportion was able to mature during the winter. A number of the riper spores may also have fallen off during the process of sampling. Possibly, teleutospores developing and passing the winter under natural conditions would show a higher percentage of germination, and would also germinate over a longer period.

(*b*) *Infection experiments with teleutospores*

Numerous attempts have been made to infect rose bushes with rust by means of the teleutospores. In addition to the more usual method of hanging leaves on or over the plants, bushes have been atomized with spore suspensions and infected leaves have been placed on the soil. Inoculated plants have been kept in the moist chamber for varying periods or placed immediately on the bench. In no case has any infection taken place though, in experiments made during May 1935, spores from the leaves used for inoculation showed a small percentage of germination.

The writer has been informed by Dr Deacon of Norwich that he has seen teleutospores adhering to the stems of rose bushes by their mucilaginous pedicels. In the germination experiments described above only spores floating on water germinated and it may be hazarded that, in nature, a film of rain water may persist in cracks in the bark or between bud scales long enough to enable spores there lodged to germinate and to infect the plant. Dr Deacon's observations suggest that the use of a winter wash may be of value in preventing this source of infection. Caeomata may also occur on leaves but, so far, the method by which this type of infection is brought about has not been elucidated.

(*c*) *Perennial mycelium*

If, as seems likely, the teleutospores only germinate during early summer, the mycelium must be capable of surviving the winter in the stem since caeomata have been observed as early as 28 March. These caeomata continue to produce spores for some weeks but, in most cases, the branch is killed and the fungus dies. A number of pot plants of Kokulensky's *canina* developed caeomata in 1935, and were segregated and kept under observation. In all cases except one the infected branches were killed and, in the following year, the plants remained healthy. In

one plant, a second zone of caeomata developed in 1936 below the portion of stem infected in the previous year. This plant again produced a diseased bud in the autumn of 1937 on the same branch and below the previous lesions. At no time did the leaves of the plant show any infection, so that the mycelium must have travelled to a small extent in the tissues of the host. There seems, however, to be no evidence of systemic infection such as has been found in the Blackberry in America (Dodge, 1923). There seems little reason, therefore, to fear the passage of rust from an infected stock to the rose grafted on it. Stems showing caeomata should be cut out as soon as observed since it appears that this spore stage is, probably, the most important in starting an epidemic of the disease.

Summary

1. Cross-inoculation experiments have shown that *Rosa laxa* and certain varieties of *R. canina* are not attacked by *Phragmidium mucronatum* from the cultivated rose, and that the rose is resistant to certain forms of the rusts on briars.

2. These differences are correlated with differences in the size of the teleutospores.

3. The teleutospores of *P. mucronatum* require exposure to winter conditions before they will germinate.

4. The mycelium of the rust may persist for several years in the stem but there is no evidence of systemic infection.

The writer is indebted to Mr Courtney Page for supplying material and for much valuable information, and to Dr W. F. Bewley for helpful criticism and advice.

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SOIL CONDITIONS AND THE TAKE-ALL DISEASE OF WHEAT

III. DECOMPOSITION OF THE RESTING MYCELIUM OF *OPHIOBOLUS GRAMINIS* IN INFECTED WHEAT STUBBLE BURIED IN THE SOIL

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(With Pl. XXVIII and 3 Text-figures)

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I. Introduction

In the first two papers of this series (Garrett, 1936, 1937), the relation of soil conditions to the subterranean spread of *Ophiobolus graminis* along the roots of wheat plants was examined; evidence was presented in support of the hypothesis that advance of the fungus was mainly controlled by the accumulation of respiratory carbon dioxide in the immediate neighbourhood of the root. On this hypothesis, the antagonism of the other soil micro-organisms to the parasitic activity of *Ophiobolus* would be represented chiefly by their contribution to soil respiration which, in turn, would raise the concentration of carbon dioxide in the neighbourhood of the root.

Soil conditions, however, affect also the second phase in the life history of *Ophiobolus*, viz. the decline in viability of its resting mycelium in the infected root and stubble residues left in the soil after harvesting the crop. The conditions of temperature, moisture and aeration which at one time

[1] The greater part of this work was carried out during the tenure of a Leverhulme Research Fellowship.

favour the rapid subterranean advance of the fungus along the roots of the growing crop may, at another time, bring about its rapid disappearance from infected plant residues buried in the fallow soil. Biological control of *Ophiobolus* by the other soil micro-organisms is exercised more, perhaps, through the direct decomposition of its resting mycelium than through the retarding effect of soil respiration upon its activity in the parasitic phase.

It has been suggested (Garrett, 1938*a*) that this alternation between an active parasitic phase on and in the underground parts of the host plant, and a resting or declining phase in the soil may be a comparatively general characteristic of the more highly specialized root-infecting fungi. Such fungi appear to be confined to existence upon their host plants, not through inability to live as saprophytes, since the majority are readily cultured both upon the ordinary laboratory media and upon sterilized soil, but on account of the rigorous competition for existence upon the decomposing organic matter of the soil. The useful distinction between obligate parasites and facultative parasites is, thus, of limited value when applied to the soil-borne fungi; to the small class of obligate parasites, such as *Plasmodiophora*, which cannot be cultured for apparently nutritional reasons, must be added a larger class behaving as obligate parasites in the soil, for reasons of biological competition.

The effect of soil conditions upon the incidence and distribution of the take-all disease of wheat is thus a problem which will not be solved before both phases in the life history of the fungus have been studied adequately: a preliminary investigation of the resting phase has now been completed, and is presented in this paper.

The decomposition of dried and powdered fungus mycelium by the soil microflora has been studied by Heck (1929), Jensen (1932), and Norman (1933). The present work appears to be the first reported on the decomposition of the resting mycelium of a parasitic fungus in the soil. The "soil plate" method, devised by Cholodny (1930), and used by Conn (1932), Jensen (1934–6) and others, has demonstrated very convincingly the rapid decomposition of vegetative fungus mycelium in the soil. The addition of such a supplement as rye-grass meal or dried blood to the soil results in a rapid multiplication of the soil fungi, the best development of which on the Cholodny slides is seen after a period of some 7–10 days at a temperature of 15–20° C.; after some 10–14 days, the fungus mycelium begins to disappear comparatively rapidly from the slides. The relation between bacteria and the decomposing hyphae is especially well seen; the sites of past hyphae on the slides are frequently

marked out by the almost continuous development of bacteria. A detailed study of the parasitism of the common soil-inhabiting fungus *Trichoderma lignorum*, upon another soil fungus, *Rhizoctonia solani*, has been made by Weindling and his collaborators (1932–6). No apology need therefore be made for the working hypothesis to be adopted in this paper, viz. that the disappearance of the resting mycelium of *Ophiobolus* from infected wheat stubble is due primarily to its direct decomposition by the soil microflora.

II. Methods

The experiments to be described have been made with artificially infected wheat stubble; the artificially infected material has the advantages over the naturally infected stubble of greater uniformity, and of being obtainable in desired amount and in a fresh condition for experiments at any time of the year. It is intended, however, to confirm all major conclusions by corroborative experiments with the natural material. The general plan of the experiments has been as follows. The pieces of artificially infected wheat straw, each some 2·5 cm. in length and thoroughly impregnated with *Ophiobolus* mycelium, have been buried in the different experimental soils in lots of 50; glass tumblers of the type previously used (Garrett, 1936) have again been employed as soil containers. Every fortnight, a sample of two tumblers, containing 100 straws in all, has been taken from each batch of soil, and the straws tested for viability of the contained *Ophiobolus* mycelium. A simple cultural test for viability, i.e. by surface sterilization of the straw and plating out on an agar medium, has not, unfortunately, been feasible. *Ophiobolus*, though not a slow-growing fungus, is easily suppressed on an agar medium by other fungi present in the infected plant tissue. The wheat seedling itself has, therefore, been adopted as the ideal selective medium for *Ophiobolus*. The infected straws have been washed free of soil, split slightly, and a wheat seed inserted in each. The straw plus seed has then been planted in sand, which affords ideal infection conditions for *Ophiobolus* (Garrett, 1936). The percentage of straws containing viable *Ophiobolus* mycelium has then been given by the number of infected wheat seedlings eventually obtained. Methods and materials employed in these experiments may now be described in more detail.

Preparation of the infected straw was made as follows. From a complete wheat straw, the nodes were cut out with scissors, each with 2–2·5 cm. of internode straw attached. One cut was made immediately below the node, the other 2–2·5 cm. above it; the internode straw consisted of the true stem together with the enclosing leaf-

sheath. Inclusion of the comparatively resistant node served to hold the straw together during the subsequent period of decomposition in the soil. A preliminary sorting of the straws was made before cutting, and those of external diameter less than 3–4 mm. were rejected. The batch of cut straws for an experiment was first boiled for 3 hr. in a decoction of string beans at the rate of 1 lb. of string beans in 4 l. of tap water to every 4000 straws. After being left overnight in the decoction, the wet straws were allowed to drain for a brief period, and were then sterilized in lots of 1000 or so in 2 l. Erlenmeyer flasks, for a period of $1\frac{1}{2}$ hr. at $1\frac{1}{2}$ atm. in the autoclave. Each flask of straws received as inoculum some 300 g. of a freshly grown culture of *Ophiobolus* on sand +3% corn-meal medium, the contents of a single 500 c.c. Erlenmeyer culture flask. The finely dispersed corn-meal-sand inoculum was well shaken up with the straws, which thus received a simultaneous and uniform inoculation. The flasks of inoculated straws were generally incubated for a period of about 1 month at 25° C. After this period of incubation, the cells of the straws were thoroughly impregnated with *Ophiobolus* mycelium. The nitrogen content of the infected straws after incubation averaged over several experiments some 0·4% of dry matter. At the conclusion of the incubation period, the straws were washed free from the adhering remains of the sand and corn-meal inoculum over a sieve, and counted in lots of fifty into small envelopes.

Soils for the experiments were air-dried shortly before use, and passed through a 1·8 mm.-mesh sieve (14 meshes to the inch). Each batch of soil was thoroughly mixed before use, and the moisture capacity at saturation determined by the perforated box method of Keen & Raczkowski (1921). Tobacco tins 2·25 cm. in depth and of some 200 c.c. capacity were found convenient for this purpose. Since the saturation capacity varies considerably with the physical state of aggregation of the soil, it was found essential to make a fresh determination for each batch of air-dried soil. The glass tumblers used as soil containers were 8·5 cm. in height by 6·75 cm. internal diameter, and were filled with dry soil to a capacity of some 180 c.c. (representing a depth of 5 cm. of dry soil). An addition of 1 g. of fertilizer per tumbler represents a dressing of 1 ton 2·5 cwt. per acre mixed with the top 2 in. of soil, or one of 3 tons 7·5 cwt. per acre mixed with the top 6 in. of soil.

In preparation for an experiment, the weight of 180 c.c. of air-dry soil was determined for each soil, and this amount weighed out per tumbler for each series. The volume of water required to bring the moisture content of each soil to 50% or other desired degree of saturation was next calculated. All tumbers had been previously numbered with their own weights, and the stock arranged in weight classes, so that the heavier tumblers could be employed for the lighter soils and vice versa. It was thus generally possible to bring some 500 tumblers, constituting a single experiment, to not more than two final weights, which greatly facilitated the periodical waterings. The supplements to be added to each soil series, if insoluble or incompletely soluble, were mixed beforehand with the air-dry soil, but if soluble were added as a solution at the time of setting up the experiment. The experiments were generally designed to run for a period of 18 weeks, so that 18 tumblers were required for fortnightly samplings in duplicate from each soil.

In setting up an experiment, the tumblers were filled as follows. The 50 straws were tipped into the bottom of the tumbler and some three-quarters of the air-dry soil added; the top of the tumbler was closed with the palm of the hand, and straws and dry soil mixed up by brisk rotatory shaking. The remaining quarter of dry soil was

added finally to cover any straws lying on top. After the 18 tumblers of one series had thus been filled with soil, the correct volume of distilled water (or solution) to bring the soil to 50% (or percentage desired) saturation was added from a measuring cylinder. If the water was added to the centre of the soil in each tumbler, i.e. at one spot only, and not too quickly, no trouble was experienced with air-locks, and a rapid and uniform percolation resulted. The straws helped to keep the soil more open and thus, no doubt, assisted percolation of the water; it was not found necessary to continue with the more cumbersome method of filling and watering the tumblers as employed in the previous work (Garrett, 1936). By 48 hr. after filling, the distribution of moisture was comparatively even, as judged by soil colour; indeed, the difficulties of moisture distribution were largely avoided by working with these small quantities of soil. Only where moisture contents in the neighbourhood of 30% saturation were employed was it found desirable to mix soil and water by hand in a basin before filling into the tumblers. The tumblers were brought to the appointed final weight by the addition of some 100 g. of a mulch of moist sand (at 50% saturation).

An experiment comprised some 20–30 series of eighteen tumblers each, and the setting up was spread out over 2 days, which made the subsequent work of sampling more easily managed. The tumblers were kept on trestle tables in the open laboratory, the temperature of which (recorded by thermograph) varied from 15–22° C. during winter and summer, with an average temperature of approximately 18° C. Tumblers were arranged not in series but randomized on the tables, to cancel out any possible place effect. The sand mulch greatly reduced water loss by evaporation; this was made good on a box balance once a week. Tumblers of pure sand lost water much more rapidly, and these were generally watered in mid-week from a constant-delivery pipette on a rubber hose.

Sampling was done at fortnightly intervals from 2 to 18 weeks. The ball of soil from each tumbler was knocked out on to a sieve, and the infected straws sieved free of soil in water. Each straw was split halfway down from the upper (or internode) end with two cross-cuts of a scalpel, and a Little Joss wheat seed inserted. The fifty straws from each tumbler, each enclosing a wheat seed, were planted in stencil-made holes in a wooden seedling flat of pure sand, in five rows of ten each, 3 cm. between seedlings and 4 cm. between rows. The sampling and planting was done by three persons, the first washing out and splitting the straws, the second inserting the wheat seeds, and the third doing the actual planting in the flats of sand. The flats were stacked in the laboratory (mean temperature 18° C.) for 1 week, until seedlings were emerging, and were then transferred to the glasshouse (20–30° C.) for a further 2 weeks' growth of the young plants. Seedlings were washed out from the flats of sand 3 weeks after planting. No nutrient solution was found necessary for this limited period of growth, and the sand was steamed and used repeatedly.

Determination of infection was made by inspection of each wheat seedling floated out in water over a white-enamelled tray. The binocular dissecting microscope was used at first to confirm the presence of the runner hyphae of *Ophiobolus*, but it was soon found that proximal discoloration of the seedling roots was a reliable criterion of the presence of the fungus, under the standardized conditions of these experiments. This method is not claimed to be an infallible test for the presence or absence of viable *Ophiobolus* mycelium in the infected straws. But sand is a rooting medium more favourable for infection than any naturally occurring field soil (Garrett, 1936),

and the roots of the seedling are enabled to explore the infected straws very effectively (see Pl. XXVIII, which shows examples of severely infected and healthy seedlings respectively; all gradations between the two are naturally found). Moreover, at the time of washing out of the seedlings, infection had generally advanced some 4–5 cm. or more along the roots, so that nothing would have been gained by a longer growing period. Since the spread of the fungus from one seedling to another can occur only through root contact (Garrett, 1936), the 3 weeks' growing period represented a satisfactory compromise, giving ample time for the development of infection, but not enough for appreciable spread of the fungus from one seedling to another by root contact.

It will be understood that a seedling having visible discoloration on the proximal part of one only, or more, of its roots was regarded as infected by *Ophiobolus*; the straw through which it had grown was therefore recorded as still containing viable mycelium of the fungus. Figures for percentage viability of *Ophiobolus* given from the following experiments are thus based on the wheat seedling test of 100 pieces of infected straw, derived from a sample of 2 tumblers taken fortnightly from each soil series. It must be emphasized that percentage viability of the fungus as indicated at any time by this test is necessarily greater than the total percentage of mycelium still viable in the mass of 100 infected straws regarded as a whole; the terms "50% viability" and "100% viability", where subsequently used for the sake of brevity, must therefore be taken as referring strictly to the percentage of straws containing viable fungus.

III. EXPERIMENTAL

The experiments will be numbered serially and described in chronological order:

Exp. I. The following series of experimental soils were set up:

Series 1–4. Slough soil air-dry and at moisture contents of 30, 50 and 80% of saturation, respectively. The soil was obtained from the Biological Field Station of the Imperial College of Science and Technology at Slough,[1] and is similar to that used in the previous work with *Ophiobolus*. The air-dry stock prepared for this experiment had a saturation capacity of 60 c.c. per 100 g. of air-dry soil; the *p*H value was determined by quinhydrone electrode as 7·2. The soil at 30% saturation was mixed with water by hand before weighing out into the tumblers.

Series 5–8. Inoculated sand air-dry and at moisture contents of 30, 50 and 80% saturation respectively. The saturation capacity of the sand was determined as 22 c.c. per 100 g. of air-dry sand. The series at 30, 50 and 80% saturation were inoculated with the microflora of Slough soil, prepared by shaking up 200 g. of moist screened soil with 1 l. of water; the suspension was allowed to settle for 1 min., and then decanted and used for the watering of the sand. Unfortunately, the heaviness of the inoculation thus increased with the moisture content of the sand, but the possible importance of actual numbers of organisms added was not appreciated when the experiment was set up. The series at 30% saturation was mixed with water by hand.

Series 9 *and* 10. Inoculated sand and Slough soil, respectively, +3% of rye-grass meal (nitrogen content 1·2% of dry matter).

[1] By courtesy of Prof. W. Brown.

Series 11 *and* 12. Slough soil treated with 0·3% of sulphuric acid and with 0·25% of calcium hydroxide, respectively, by weight of the air-dry soil. The soils were mixed with the respective chemicals at a moisture content of 40% saturation, incubated in loosely-covered glass containers for 15 days at laboratory temperature (18° C.), and then air-dried before use. The *p*H values of the acid-treated and limed soils were electrometrically determined as 5·8 and 8·3, respectively.

Series 13 *and* 14. Series 13 was Slough soil at 50% saturation maintained under improved conditions of aeration in 4-in. unglazed flower pots, of approximately the same capacity as the glass tumblers. Additional water had to be added (22 c.c. per pot) to compensate for that taken up by the porous walls. Series 14 was Slough soil at 50% saturation, in tumblers kept in a loosely-covered glass jar inside an incubator maintained at 23° C., i.e. under very still and uniform conditions.

Series 15. Infected straws kept air-dry in the absence of soil in empty glass tumblers.

The results of this experiment are given in Table I and are shown graphically in Text-fig. 1.

Table I. *Percentage viability of* Ophiobolus *resting mycelium in infected straws*

Soil series	Weeks 2	4	6	8	10	12	14	16	18
No. 1, soil, dry	100	100	100	100	100	100	100	100	100
,, 2, soil, 30% sat.	100	98	99	77	72	26	13	9	1
.. 3, soil, 50% sat.	100	86	90	74	61	47	22	36	4
.. 4, soil, 80% sat.	100	97	92	76	64	24	44	19	58
,, 5, inoc. sand, dry	100	100	100	100	100	100	100	100	100
,, 6, inoc. sand, 30% sat.	100	100	98	85	100	84	100	76	39
,, 7, inoc. sand, 50% sat.	100	100	100	99	96	90	56	50	3
,, 8, inoc. sand, 80% sat.	100	98	87	94	97	27	59	51	18
,, 9, inoc. sand, +3% rye-grass	100	97	84	6	4	0	—	—	—
,, 10, soil + 3% rye-grass	100	49	21	2	0	0	—	—	—
,, 11, soil + 0·3% sulphuric acid	100	86	82	17	2	0	—	—	—
,, 12, soil + 0·25% calcium hydroxide	100	86	63	21	1	0	—	—	—
,, 13, soil at 50% sat. in 4 in. flower pots	100	96	81	58	50	45	5	19	0
.. 14, soil at 50% sat. in closed incubator	100	95	92	74	68	77	71	71	47
,, 15, straws kept air-dry in empty tumblers	100	100	100	100	100	100	100	100	100

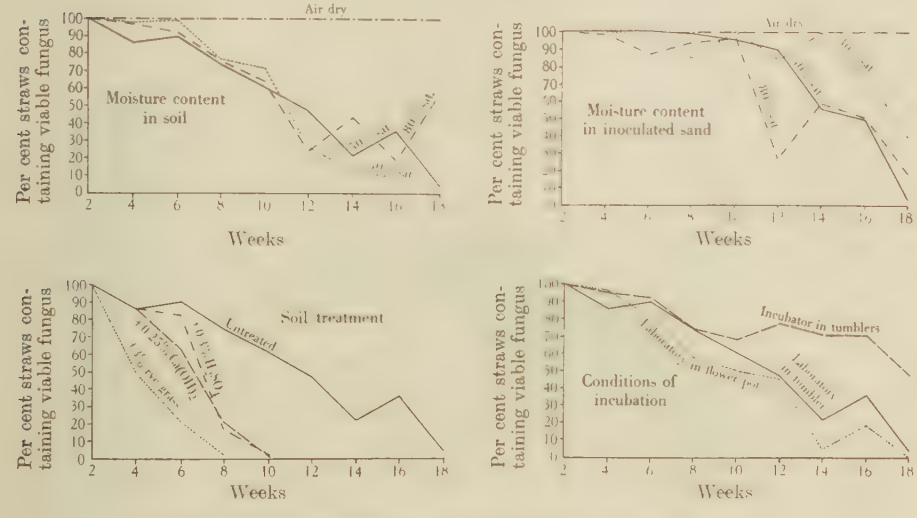
The following conclusions may be drawn:

(i) The viability of the fungus persisted apparently undiminished (i.e. as shown by this test) throughout the experimental period of 18 weeks in dry air, sand and soil.

(ii) In the moist soils, variation in moisture content over the range 30–80% of saturation did not appreciably affect the decline of the fungus, either in Slough soil or in inoculated sand. The decline was less regular at 80% saturation than at the two lower moisture contents.

(iii) In the three Slough soil series, 50% loss of viability occurred at 10–12 weeks, as against 16–18 weeks in the three inoculated sand series. It appears that the better aeration in the inoculated sand series did not compensate for the much lower numbers of micro-organisms initiating decomposition in this series.

(iv) The addition of 3% of rye-grass meal greatly accelerated loss of viability both in Slough soil and in the inoculated sand. This may be attributed tentatively to increase in the numbers of soil micro-organisms through multiplication on the added organic material and, thus, to a more rapid attack on the *Ophiobolus* mycelium in the infected straw.



Text-fig. 1. Decline in viability of the fungus in the soils of Exp. I.

(v) Pre-treatment of the soil both with lime and with sulphuric acid accelerated the disappearance of *Ophiobolus*. Discussion of this result will be postponed for the production of further data in Exp. II.

(vi) Loss of viability in the flower-pot series appears to have proceeded at a slightly but uniformly greater rate than in the corresponding tumbler series. In the series maintained in a lightly covered glass vessel in the incubator, loss of viability at first kept pace with that in the control series kept in the open laboratory, but then slowed down abruptly after 8 weeks. Whereas 50% loss of viability occurred in the open laboratory control at 12 weeks, it was deferred until 18 weeks in the incubator series. It may be suggested that improved aeration in the flower pot series has

increased microbiological activity, and hence loss of viability of *Ophiobolus*, whereas decreased aeration in the incubator series has correspondingly reduced it. The maintenance of uniform conditions in stored soil is known to lead to reduction of microbiological activity to a comparatively low level (Waksman & Starkey, 1923), and it is probable that other factors besides a retarded gas exchange in the incubator series have contributed to the observed result.

Exp. II. All soils in this experiment were started and maintained at a moisture content of 50% saturation; the following series were set up:

Series 1–8. Different soil types.

Series 1. Carbello, *p*H 4·9, an organic loam.

Series 2. Tunstall, *p*H 5·5, a sandy heath soil.

Series 3. Rothamsted, *p*H 6·8, a clay loam.

Series 4. Woburn, *p*H 7·2, a light loam, previously well limed.

Series 5. Slough, *p*H 7·2, a medium loam.

Series 6. Downham, *p*H 7·5, a black fen soil.

Series 7. Whitchurch, *p*H 8·1, a clay loam on chalk subsoil.

Series 8. Aldeburgh, *p*H 8·1, a sandy heath soil, well limed.

Series 9. Slough soil, kept in refrigerator at 2–3° C.

Series 10 *and* 11. Slough soil, pre-treated with 0·3% sulphuric acid and with 0·25% calcium hydroxide, respectively, exactly as in Exp. I, to give soils of *p*H values 5·8 and 8·2, respectively.

Series 12. Slough soil, steamed moist for 3 hr., air-dried and reinoculated by mixing with a tenth part by weight of the unsterilized soil.

Series 13–17. Slough soil with 3% by weight of the following organic supplements, starch, rye-grass meal, corn-meal, rape dust and dried blood.

Series 18–21. Slough soil adjusted to different C/N ratios by addition of 5 g. glucose per tumbler, corresponding to 2·5 g. glucose or to 1 g. carbon per 100 g. dry soil, together with variable amounts of ammonium carbonate to give C/N ratios of 100, 50, 10 and 5, respectively.

Table II. *pH values of soils from Exp. II*

Soil series	Weeks 2	4	6	8	10	12	14	16	18
No. 5, untreated Slough	7·2	7·1	7·1	—	—	—	—	—	—
,, 13, +3% starch	7·4	7·5	7·3	7·5	7·6	7·5	7·7	7·6	7·4
,, 14, +3% rye-grass	7·3	7·2	7·3	7·3	7·3	7·3	7·2	—	—
,, 15, +3% corn-meal	7·3	7·4	7·2	7·2	7·2	7·1	7·1	6·9	6·9
,, 16, +3% rape dust	7·0	5·0	4·8	5·0	4·9	4·8	4·9	4·9	4·9
,, 17, +3% dried blood	7·7	5·7	5·0	5·0	4·7	4·7	4·5	4·4	4·5
,, 18, C/N 100	7·3	7·0	7·2	7·3	7·3	7·3	7·0	7·1	7·1
,, 19, C/N 50	7·2	6·3	6·6	6·7	6·8	6·7	6·7	6·6	6·5
,, 20, C/N 10	8·3	5·0	5·1	5·2	5·4	5·3	5·5	5·4	5·3
,, 21, C/N 5	8·6	7·2	5·9	5·2	4·9	4·9	4·9	5·0	5·0

The *p*H values of soils to which the various organic supplements had been added, and of those with adjusted C/N ratios, were determined periodically by means of the quinhydrone electrode, and are given in Table II.

It will be observed that a considerable fall in *p*H value eventually follows the addition of rape dust and of dried blood, both with a high nitrogen content, and that this occurs also in series 20 and 21 with C/N ratios of 10 and 5, respectively. This increase in soil acidity is to be attributed to the rapid progress of nitrification in the absence of a sufficient reserve of base for neutralization of the nitric acid produced.

The results of this experiment are given in Table III and Text-fig. 2. The following conclusions may be drawn from the data there presented:

Table III. *Percentage viability of* Ophiobolus *resting mycelium in infected straws*

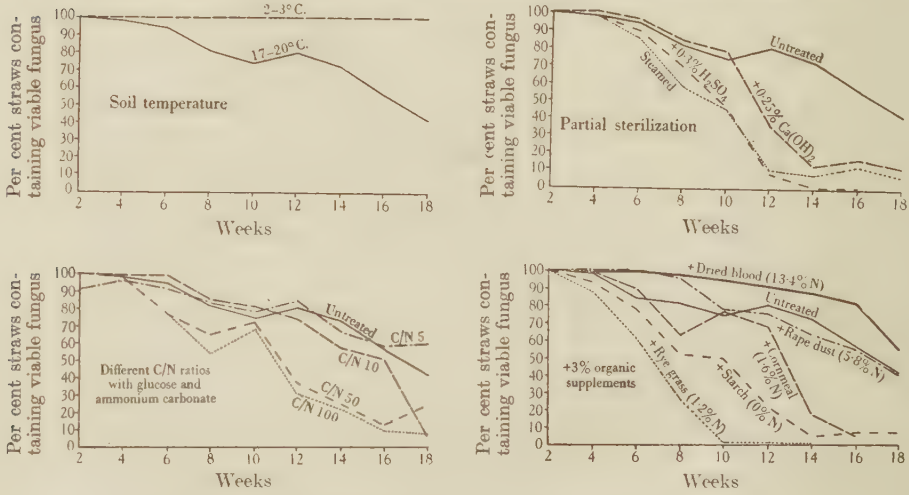
Soil series	Weeks								
	2	4	6	8	10	12	14	16	18
No. 1, Carbello	100	100	90	88	87	79	81	69	51
,, 2, Tunstall	100	98	90	78	78	64	55	34	3
,, 3, Rothamsted	100	99	83	31	13	22	5	1	0
,, 4, Woburn	100	99	88	83	57	77	43	35	16
,, 5, Slough	100	98	94	81	74	80	72	56	41
,, 6, Downham	100	92	95	93	89	80	67	51	25
,, 7, Whitchurch	100	95	88	35	22	9	12	0	1
,, 8, Aldeburgh	100	97	91	83	84	86	78	60	24
,, 9, in refrigerator	100	100	100	100	100	100	100	100	100
,, 10, +0·3% sulphuric acid	100	98	89	69	46	8	1	1	0
,, 11, +0·25% calcium hydroxide	100	100	96	84	78	36	13	17	12
,, 12, steamed and reinoculated	100	98	85	58	45	11	8	13	7
,, 13, +3% starch	100	93	78	52	49	22	5	7	7
,, 14, +3% rye-grass	100	88	61	26	1	1	1	—	—
,, 15, +3% corn-meal	100	99	89	63	76	67	17	5	0
,, 16, +3% rape dust	100	100	100	95	77	75	63	54	40
,, 17, +3% dried blood	100	99	99	97	94	90	86	80	54
,, 18, C/N 100	100	98	77	54	68	31	22	9	7
,, 19, C/N 50	100	98	77	65	72	37	25	13	24
,, 20, C/N 10	100	99	99	85	81	74	57	50	6
,, 21, C/N 5	91	96	91	83	78	84	66	58	59

(i) From an examination of rates of decline in the different field soils of series 1–8, no correlation can be made out between rate of decline and *p*H value of the soil (which affects so markedly the parasitic activity of *Ophiobolus*). Two soils, those of Rothamsted and Whitchurch, stand out in marked contrast to the remainder in virtue of the high rate of decline in them. These two soils are alike in being clay loams, and the heaviest soils in the series. It scarcely seems likely that reduced aeration in these soils can be the reason for the rapid loss of *Ophiobolus* viability in them, since improved aeration seemed to increase rate of decline in the flower-pots of Exp. I, whilst in Exp. III this is confirmed; it will, further, be shown that *Ophiobolus* declines more slowly in a waterlogged soil than in one at medium moisture content. From the results of other workers (Waksman, 1931), it might be expected that numbers of bacteria and

actinomycetes would be considerably higher in these two heavy soils than in the six remaining soils.

(ii) No loss of viability was revealed by the test throughout the duration of the experiment in the series kept at 2–3° C. in the refrigerator.

(iii) A marked increase in rate of decline of viability occurred as a result of pre-treatment of Slough soil with sulphuric acid and with lime, as in Exp. I, and also as a result of partial sterilization by steam and subsequent reinoculation of the soil. These results, together with the apparent absence of any direct relation between soil *p*H and rate of decline in series 1–8 of this experiment, suggest that the action of the chemicals has



Text-fig. 2. Decline in viability of the fungus in the soils of Exp. II.

produced a partial-sterilization effect (Hutchinson & MacLennan, 1914), similar to that of steaming, and that the enhanced power of these three soils to decompose *Ophiobolus* mycelium is due to the sudden rapid increase in microbiological activity as a result of partial sterilization.

(iv) The effectiveness of the organic supplements added to the soil in series 13–17 in accelerating decline of *Ophiobolus* appeared to fall off with increasing nitrogen content above 1·2% of dry matter (rye-grass meal). In Table IV the nitrogen contents of the different organic supplements are set out against the approximate times for 50% decrease in viability of the fungus in these series. It will be observed that rape dust produced no acceleration and dried blood a retardation in decline of the fungus.

Table IV. *Nitrogen contents of organic supplements in relation to the viability of* Ophiobolus

	Starch	Rye-grass	Corn-meal	Rape dust	Dried blood	No treatment
Time in weeks to 50% decrease in viability of fungus	9	6	13	17	18+	17
Nitrogen content of added organic matter as % dry matter	0	1·2	1·6	5·8	13·4	—

(v) It is unfortunate that a glucose alone treatment was not included in series 18–21 with variable C/N ratio. The most rapid decline of the fungus occurred in series 18 with C/N ratio 100, and the effectiveness of the glucose appeared to be progressively reduced by increasing amounts of nitrogen up to a C/N ratio of 5. In this series, decline was no more rapid than in the untreated control soil, and there is indeed evidence of an actual retardation due to the treatment at 18 weeks.

Exp. III. The contrast observed in the previous experiment between the action of rye-grass meal and that of dried blood seemed so striking as to merit further investigation. In this experiment, accordingly, four rather different soils were selected to receive dressings of rye-grass meal at three different levels, and of dried blood at two different levels. All soils were started and maintained at a moisture content of 50% saturation.

The rye-grass meal was applied at the rates of 6, 1 and 0·2 g. per tumbler, and the dried blood at the rates of 1 and 0·2 g. per tumbler. These amounts were added per 180 c.c. of dry soil to the different soils, since owing to their different "apparent densities" (Robinson, 1936), volume seemed to afford a better basis of equivalence than weight. Since dried blood is very rapidly decomposed in the soil, it seemed better to omit the dressing of 6 g. per tumbler, as likely to lead to excessive accumulations of soluble substances which might prove toxic to the *Ophiobolus* mycelium, and produce misleading results.

The four soils employed were as follows:

Bridgham: a very light sandy soil of the "Breckland" type from Norfolk, containing some 7% of calcium carbonate, and giving a *p*H value of 7·8.

Woburn: a typical light loam from the Woburn Experiment Station, of *p*H 6·4.

Downham: a black fen soil from Norfolk, of *p*H 7·8.

Slough: as used in previous experiments but this batch of soil was somewhat acid, giving a *p*H value of 6·2.

The drift in *p*H values in the differently treated soils was followed by means of the quinhydrone electrode (Table V).

Table V. *p*H *values of soils from Experiment III*

Soil series	Weeks 2	4	6	8	10	14
Series 1–6, Bridgham:*						
No. 1, untreated	7·8	7·8	7·9	—	—	—
,, 2, +0·2 g. rye-grass	7·8	7·9	7·9	—	—	—
,, 3, +1 g. rye-grass	7·8	7·9	7·9	—	—	—
,, 4, +6 g. rye-grass	7·9	8·1	8·1	—	—	—
,, 5, +0·2 g. dried blood	7·5	7·6	7·9	7·6	7·5	7·9
,, 6, +1 g. dried blood	7·3	7·4	7·5	7·5	7·4	7·6
Series 7–12, Woburn:						
No. 7, untreated	6·4	6·3	6·4	6·6	6·4	6·6
,, 8, +0·2 g. rye-grass	6·2	6·3	6·2	6·5	6·4	6·6
,, 9, +1 g. rye-grass	6·4	6·5	6·4	6·5	6·5	6·9
,, 10, +6 g. rye-grass	6·6	6·6	6·8	6·6	6·7	7·1
,, 11, +0·2 g. dried blood	5·7	5·8	6·0	6·2	6·1	6·6
,, 12, +1 g. dried blood	6·0	4·9	4·6	4·6	4·5	4·8
Series 13–18, Downham:						
No. 13, untreated	7·5	7·7	7·8	7·8	7·8	7·8
,, 14, +0·2 g. rye-grass	7·5	7·7	7·8	7·8	7·8	7·8
,, 15, +1 g. rye-grass	7·6	7·8	7·7	7·9	7·8	7·8
,, 16, +6 g. rye-grass	7·7	7·8	7·8	7·9	7·8	7·9
,, 17, +0·2 g. dried blood	7·3	7·5	7·6	7·6	7·6	7·7
,, 18, +1 g. dried blood	7·1	7·2	7·3	7·5	7·4	7·5
Series 19–24, Slough:						
No. 19, untreated	6·1	6·2	6·2	6·3	6·3	6·6
,, 20, +0·2 g. rye-grass	6·2	6·2	6·0	6·4	6·0	6·5
,, 21, +1 g. rye-grass	6·2	6·4	6·4	6·1	6·1	6·5
,, 22, +6 g. rye-grass	6·6	6·5	6·3	6·3	6·4	6·7
,, 23, +0·2 g. dried blood	5·7	5·9	5·8	5·8	5·9	6·5
,, 24, +1 g. dried blood	6·0	5·2	4·5	4·5	4·6	4·8

* Quinhydrone electrode determinations with the Bridgham soil gave *p*H values showing drift in the alkaline direction attributable to manganese error (Crowther & Heintze, 1930). *p*H determinations of the Bridgham soils are, therefore, only approximate.

As a subsidiary experiment, a parallel series with Slough soil at 50% saturation was set up in 4 in. flower pots as in Exp. I, but here the sand mulch was omitted, further to improve soil aeration. A second batch of Slough soil was waterlogged in tumblers, and maintained at 100% saturation throughout the experiment.

The complete results of this experiment are set out in Table VI, and are illustrated in Text-fig. 3.

The following conclusions may be drawn:

(i) The decline of the fungus was considerably more rapid in the two better soils, Slough and Downham, than in the two poorer soils, Woburn and Bridgham.

Table VI. *Percentage viability of* Ophiobolus *resting mycelium in infected straws*

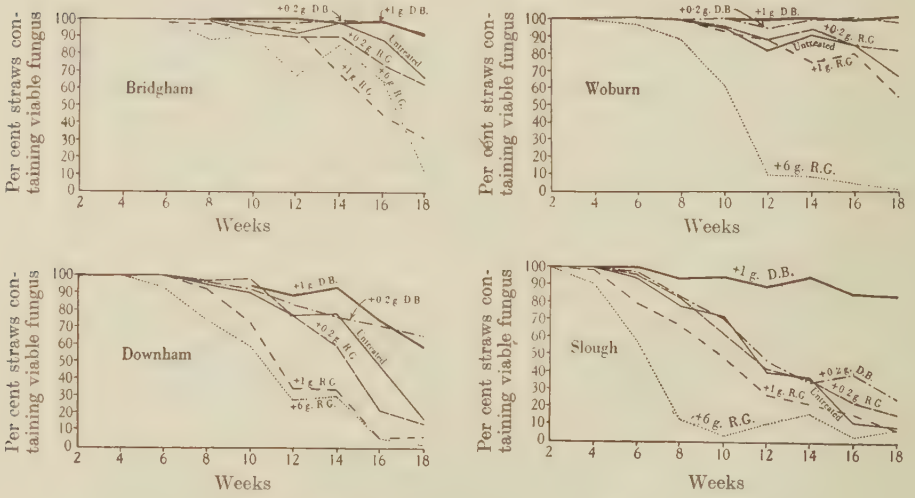
Soil series	Weeks 2	4	6	8	10	12	14	16	18
Series 1–6, Bridgham:									
No. 1, untreated.	100	100	100	100	96	92	98	88	66
,, 2, +0·2 g. rye-grass	100	100	100	99	92	90	90	74	62
,, 3, +1 g. rye-grass	100	100	98	97	96	94	68	45	31
,, 4, +6 g. rye-grass	100	100	98	88	90	67	85	64	12
,, 5, +0·2 g. dried blood	100	100	100	100	98	98	99	98	92
,, 6, +1 g. dried blood	100	100	97	99	100	100	98	99	91
Series 7–12, Woburn:									
No. 7, untreated	100	100	100	99	94	81	90	84	66
,, 8, +0·2 g. rye-grass	100	100	98	100	95	87	93	84	81
,, 9, +1 g. rye-grass	100	100	100	99	92	87	74	79	54
,, 10, +6 g. rye-grass	100	99	96	87	60	9	8	4	1
,, 11, +0·2 g. dried blood	100	100	100	99	100	94	99	100	97
,, 12, +1 g. dried blood	100	100	100	99	100	99	100	99	100
Series 13–18, Downham:									
No. 13, untreated	100	100	100	95	90	77	78	48	17
,, 14, +0·2 g. rye-grass	100	100	100	97	98	77	59	22	14
,, 15, +1 g. rye-grass	100	100	100	92	73	35	34	6	7
,, 16, +6 g. rye-grass	100	100	93	74	59	28	30	6	2
,, 17, +0·2 g. dried blood	100	100	98	96	92	83	76	71	65
,, 18, +1 g. dried blood	100	100	100	94	94	88	93	74	58
Series 19–26, Slough:									
,, 19, untreated	100	100	94	78	72	40	37	11	8
,, 20, +0·2 g. rye-grass	100	100	96	83	63	42	36	23	16
,, 21, +1 g. rye-grass	100	99	80	67	49	27	22	16	7
,, 22, +6 g. rye-grass	100	91	58	13	3	10	16	3	7
,, 23, +0·2 g. dried blood	100	100	98	84	71	47	35	39	25
,, 24, +1 g. dried blood	100	100	100	94	95	89	95	85	84
,, 25, soil in flower-pots	100	100	97	66	24	7	5	8	—
,, 26, waterlogged soil	100	100	100	100	85	64	58	28	32

(ii) Increasing dressings of rye-grass meal produced a progressively greater effect on the decline of the fungus, but the treated soils still fell into the same order of effectiveness at all levels of treatment with rye-grass meal as the untreated soils. There was one exception caused by the unexpectedly good response of Woburn soil to the 6 g. dressing; this soil was then slightly more effective than Downham soil with the same dressing.

(iii) Both the 1 g. and the 0·2 g. dressings of dried blood retarded decline of the fungus in all four soils. The effect was most striking in the case of the 1 g. dressing with Slough soil; the 0·2 g. dressing with this soil only appeared to exert a significant effect at the two last samplings.

The poor performance of the Bridgham soil is of particular interest inasmuch as severe outbreaks of the take-all disease in crops of wheat and barley were noted during the 1937 season on land of this type that had been under sugar beet as the previous crop (Garrett, 1938*b*).

The results of the subsidiary experiment on the effect of increased aeration in 4 in. flower-pots, on the one hand, and of waterlogging, on the other, are also given in Table VI. Decline in viability of *Ophiobolus* was considerably more rapid in the flower-pots in this experiment than it was in Exp. I. The greater difference between the flower-pot series and the tumbler controls in this experiment may be partly attributed to the fact that no sand mulch was added to the flower-pots, in an endeavour still further to increase aeration. This led to more rapid drying of the soil between waterings, which were made at intervals of 3–4 days. The possibility that the rapid fluctuation in soil moisture content adversely



Text-fig. 3. Decline in viability of the fungus in four soil types of Exp. III, treated with rye-grass (R.G.) and with dried blood (D.B.), respectively.

affected the resistance of the fungus mycelium cannot be excluded; at the same time, such fluctuation would result in still further improvement in soil aeration, which would be reflected by increased microbiological activity. Fluctuating soil conditions are known to be more conducive to the microbiological efficiency of the soil as a whole (Waksman, 1931) than are very uniform and constant conditions, such as are obtained in an incubator (Exp. I). The fact that the viability of *Ophiobolus* declined more slowly in the waterlogged soil than in the soil at 50% saturation again indicates that the resting mycelium of the fungus is more tolerant of adverse physical conditions of the soil than it is of conditions favouring general microbiological activity.

In order to supplement the conclusions derived from Exp. III, a series of observations on the microflora developing in the differently treated soils was made by means of the *soil plate* method, originally devised by Cholodny (1930) for direct microscopical study of the soil flora. This method has already been applied with success by King *et al.* (1934) to a study of the biological control of *Phymatotrichum omnivorum*, causing cotton-root rot, by means of organic manures. In this experiment, the four soils Slough, Downham, Bridgham and Woburn were compared (*a*) untreated, (*b*) with addition of the 1 g. dressing of dried blood per tumbler, (*c*) with addition of the 1 g. dressing of rye-grass meal per tumbler. Tumblers were set up in duplicate; two clean microscope slides were buried vertically, some 3 cm. apart, in the dry soil of each tumbler before adding the correct amount of distilled water to bring the soil to 50% saturation. The slides were removed for examination at 10, 14 and 28 days. The balls of moist soil, containing the slides, were knocked smartly out of the tumblers; the slides were then pulled away from each face of soil in turn, as recommended by Conn (1932). After removal from the soil, the slides were air-dried and one side was cleaned with a pad of damp cotton wool. The slides were then immersed for 1 min. in the boiling staining and fixing solution, consisting of 5% erythrosin in 5% aqueous phenol, rinsed in distilled water, and air-dried. They were then stored dry, for examination at leisure under a water mount.

The Cholodny soil plate method, as originally devised, had the acknowledged disadvantages, when compared with the agar plate counting methods, of being only a qualitative method, and of offering little opportunity for the identification of the observed micro-organisms even as genera. In order to overcome the first of these two disadvantages, Jensen (1934-6) devised a method of estimating the frequency of fungus mycelium on the slides, by recording the presence or absence of hyphae in 500–550 randomized microscope fields on each Cholodny slide. He employed as his microbiological quadrat a square field of 65 μ side-length, examined under a $\frac{1}{12}$ immersion objective. His method has been modified for this experiment, by restricting examination to 50 microscope fields per slide; a circular field of approximately 315 μ diameter, obtained by use of a $\frac{1}{6}$ objective and 10× eyepiece with a body length of 200 mm., was selected; the fifty fields were examined at intervals of 1 mm. in a median longitudinal transect of the slide. The presence or absence of (*a*) fungal hyphae, (*b*) actinomycete hyphae has been recorded for each of the fifty fields examined on each slide. Since the 10-day series gave the best development of micro-organisms on the slides, this series was the

one selected for recording; the 14 and 28-day series were more rapidly examined without quantitative recording. The data given in Table VII are based on the examination of four slides from two tumblers of each soil; the following conclusions may be drawn:

(i) Only poor development of fungus and actinomycete mycelium occurred in the untreated soils, but a considerable increase in both followed the addition either of rye-grass meal or of dried blood. In the untreated soils, the best development of fungi and actinomycetes occurred in Slough soil, followed in order by that in Bridgham, Downham, and Woburn soils.

Table VII. *Percentage microscope fields showing (a) fungus mycelium and (b) actinomycete mycelium on the Cholodny slides, after a period of* 10 *days*

	Percentage occurrence of fungi			Percentage occurrence of actinomycetes		
	Untreated	+rye-grass	+dried blood	Untreated	+rye-grass	+dried blood
Slough soil	10	60	56	15	49	53
Downham soil	2	29	18	7	25	45
Bridgham soil	6	39	24	11	19	40
Woburn soil	1	25	33	2	9	22
Standard errors:						
Slough soil	±6·3	±4·2	±5·5	±2·6	±0·5	±3·8
Downham soil	±2·0	±2·4	±2·2	±0·7	±2·9	±5·7
Bridgham soil	±1·2	±7·1	±1·8	±0·7	±4·2	±6·8
Woburn soil	±0·7	±1·6	±7·6	±2·0	±3·1	±6·8

(ii) In the treated soils, the fungi developed best in Slough soil, occurring in some 60% of the fields on eight slides; in the Bridgham and Woburn soils, fungi occurred in some 30% of the fields, whilst in the Downham soil, they occurred only in some 25% of fields.

(iii) In the treated soils, the actinomycetes developed best in Slough soil, occurring in some 50% of the fields; they occurred in 35% of the fields in Downham soil, in 30% of the fields in Bridgham soil, and in only 15% of the fields in Woburn soil.

(iv) Rye-grass appears to have been scarcely more effective than dried blood in promoting fungus development; thus rye-grass was more effective than dried blood in Downham and Bridgham soils, approximately equally as effective in Slough soil, and actually less effective in Woburn soil.

(v) Dried blood, on the other hand, was definitely more effective than rye-grass in promoting actinomycete development; actinomycete mycelium was approximately twice as abundant on the dried blood slides

as on the rye-grass slides in the Downham, Bridgham and Woburn soils; in Slough soil there was no significant difference.

The observations as a whole indicate greatest microbiological activity in the Slough soil, in which, also, *Ophiobolus* disappears most rapidly. They have failed to indicate any differences in the development of fungi and actinomycetes which might explain the diverse effects of rye-grass meal and of dried blood on the rate of decline of *Ophiobolus*; the development of fungi was almost as good, and that of the actinomycetes much better, in the dried blood soils as in the rye-grass soils.

Examination of the slides left in the soils for 14 and for 28 days, respectively, supported the observations of other investigators cited above as to the rapid development and decline of the original soil microflora in the treated soils. The relationship of bacteria to the disappearing fungus hyphae was especially noticeable on the 14-day and 28-day slides; former sites of fungus hyphae were frequently marked out by the almost continuous development of small bacterial colonies. The part played by bacteria in the decomposition of fungus hyphae is especially well demonstrated by this technique.

Exp. IV. In this experiment, infected straws were buried in soil at the standard rate of 50 straws per 200 g. (=180 c.c.) of air-dry soil, and also at the rates of 10 and 200 straws, respectively, per 200 g. of soil. The "200" straw/soil ratio was arranged by burying 100 straws in 100 g. of air-dry soil per tumbler, since 100 straws has been the standard sample throughout these experiments. In this series, the straws were placed in contact, and there was then enough dry soil to fill up all the interstices and provide for a covering of soil some 1 cm. deep on top. A sample of 100 straws from each series was taken every 3 weeks to 21 weeks, instead of at the usual fortnightly intervals. The effect of the straw/soil ratio on the decline of *Ophiobolus* is shown by the results of this experiment, given in Table VIII.

Table VIII. *Percentage viability of* Ophiobolus *resting mycelium in infected straws*

Series	Weeks						
	3	6	9	12	15	18	21
No. 1, 10 straws per 200 g. soil	100	100	99	96	96	88	89
,, 2, 50 straws per 200 g. soil	100	99	99	98	91	91	84
,, 3, 200 straws per 200 g. soil	100	99	95	83	51	43	36

The more rapid decline of *Ophiobolus* in the "200" series than in the "10" and "50" series may be attributed partly, no doubt, to improved aeration of the soil by incorporation of the additional straw. It is also

possible that by increasing the number of straws, the number of organisms stimulated into activity in the soil immediately around each individual straw is also increased. Both of these factors, however, may be subordinate to a third—that of nitrogen poverty of the soil induced by crowding of the straws.

Exp. V. No experiments on the rate of dying out of *Ophiobolus* from the infected straws under sterile conditions have been performed, owing to the technical difficulties of operating with sterilized soil and effectively maintaining sterile conditions over the long periods likely to be required. A test has been made, however, of the viability of the fungus in 100 infected straws kept for a period of one year in the original culture flask in the open laboratory, i.e. under conditions of incubation precisely similar to those of the experiments with soil in tumblers. The fungus was found to be alive in all the straws, causing vigorous infection of every wheat seedling. These tests will be continued.

IV. Discussion

The results of these experiments seem to justify the thesis put forward in the introduction to this paper—that decline in the viability of the resting mycelium of *Ophiobolus* in infected wheat straw is due primarily to its natural decomposition by the other soil micro-organisms. Decline of viability in *Ophiobolus* can be correlated with microbiological activity of the soil throughout the experiments. Thus, decline is indefinitely postponed by absence of moisture in air-dry soil (Exp. I), by low temperature (Exp. II), and by the exclusion of other micro-organisms (Exp. V); it is retarded by the continued incubation of the soil under uniform conditions in an enclosed space (Exp. I). Decline is also retarded for a time under the anaerobic conditions of a waterlogged soil, so that the fungus actually disappears more quickly in soil at 50% saturation than in that at 100% saturation (Exp. III).

Decline of the fungus is accelerated, on the other hand, by addition to the soil of soluble or finely divided organic materials relatively poor or lacking in nitrogen, such as glucose, starch and rye-grass meal, which promote a rapid temporary increase in numbers and activity of the soil micro-organisms (Exps. I–III). The marked influence of pre-treating the soil with steam, lime, and sulphuric acid in accelerating decline may be attributed tentatively to the partial sterilization effect, resulting in subsequently increased numbers and activity of micro-organisms in the treated soils (Exps. I and II). Decline is most rapid in the two heaviest and richest soils of Exp. II, and proceeds more quickly in the two better

soils of Exp. III than in the two poorer soils. It is considerably more rapid in Slough soil than in inoculated sand (Exp. I), though aeration is better in the latter (improved aeration in the flower-pots of Exps. I and III produced an increase in rate of decline over that in the parallel series in tumblers). It may, therefore, be suggested that rate of decline of *Ophiobolus* is a function of the numbers and activity of micro-organisms in the soil. The rates of decline in the four soils of Exp. III seem to be related both to the initial biological state of the soil and to the amount of suitable energy material (rye-grass meal) added; this is intelligible on reflexion that numbers and activity of micro-organisms would rise more rapidly in a rich soil after addition of a given amount of energy material than those in a poor soil receiving the same dressing.

The Cholodny slides of Exp. III demonstrated the rapid development and disappearance of the original microflora resulting from the addition of 1 g. of rye-grass meal or of dried blood per tumbler to the different soils. This suggests that the rate of decline of the *Ophiobolus* mycelium in the infected straws may possibly be determined by the initial microbiological activity of the soil during the first week or two weeks of the experiment; after this period, the rate of decomposition may possibly be independent microbiologically of the surrounding soil, and affected chiefly by the non-biological conditions of incubation. It must be remembered, too, that attack proceeds from within the straw cavity as well as from the outside of the straw in these experiments.

The retarding effect apparently exercised by excess of nitrogen on the decline of *Ophiobolus* is one of the most interesting features of Exps. II and III. Both Waksman & Starkey (1923) and Winogradsky (1925) have shown that whereas substances rich in cellulose such as cereal straw and rye-grass are decomposed under aerobic conditions by fungi, actinomycetes, and bacteria, substances rich in nitrogen such as dried blood bring about much greater rises in numbers of bacteria than in those of fungi and actinomycetes. The Cholodny slides of Exp. III showed, however, nearly as great a development of fungi and twice as great a development of actinomycetes on the dried blood slides as on the rye-grass slides and, in both, the microfloral development was greatly in excess of that on slides from the untreated soils. It appears, therefore, that, under aerobic conditions, dried blood promotes a richer flora of fungi, actinomycetes and bacteria than does ground rye-grass.

More subtle differences in microfloral development according to the type of organic matter added to the soil cannot yet be excluded from consideration; it seems likely, however, that the retarding action of excess

of nitrogen on the disappearance of *Ophiobolus* may be a more direct effect. Thus an excess of nitrogen may, in some way, increase the resistance of the *Ophiobolus* resting mycelium.

A third possible explanation is suggested by the fact that fungus mycelium is itself rich in nitrogen, containing some 4–8% (Waksman, 1931); it has been successfully employed as a source of organic nitrogen in decomposition studies by Heck (1929), Jensen (1932), and Norman (1933). Since the total nitrogen content of the infected straws averaged only 0·4%, which is only about one-third the amount required for complete decomposition of normal oat straw (Norman, 1931), it may be suggested that the *Ophiobolus* mycelium itself serves as a source of nitrogen for the decomposition of the straw. The addition of organic substances rich in nitrogen, or of inorganic nitrogen in the form of ammonium carbonate as added in Exp. II may, therefore, prolong the life of *Ophiobolus* by protecting it from the nitrogen demands of the organisms engaged in decomposing the straw. This suggests that the effectiveness of rye-grass meal in promoting rapid decline of *Ophiobolus* may be due not only to increase in numbers of organisms multiplying on the added energy material but, also, to the promotion of a nitrogen scarcity in the soil around the straws. The more rapid decline of the fungus in the straws crowded at the rate of 200 per 200 g. of soil in Exp. IV may be attributed, in part at least, to the operation of this factor.

This hypothesis of nitrogen scarcity as a factor directly accelerating the disappearance of *Ophiobolus* mycelium throws a fresh light on certain observations previously difficult to explain. Throughout the experiments, it had been observed that decline of the fungus in the different series frequently failed to agree with obvious decomposition of the straw, as judged by darkening in coloration, loss of rigidity and increase in friability, etc. It had been especially remarked in Exps. I–III that straws receiving the 6 g. per tumbler dressing of rye-grass meal appeared to decompose most slowly of all, keeping their fresh appearance and rigidity the longest of any series; the same was observed of the closely crowded straws in Exp. IV. These findings, thus, exactly reverse the original expectation that the addition of a nitrogenous fertilizer, by promoting more rapid decomposition of the straw, would hasten also the disappearance of the *Ophiobolus* mycelium.

Some new problems, therefore, await solution as a result of this work. For how long after burial in the soil is the decomposition of the fungus mycelium in the infected straw dependent microbiologically on the outside soil, and how does this period vary with the individual soil? What is

the mechanism of the apparent protective effect of added nitrogen towards the *Ophiobolus* mycelium, and can this be due to preferential decomposition of a more easily available source of nitrogen? Do organic materials such as dried blood exercise an accelerating effect as energy materials for the multiplication of the soil microflora which is masked by their depressing effect as nitrogen sources? Experiments designed to answer some of these questions are now in progress. At the same time, experiments to link up these observations with the disappearance of *Ophiobolus* mycelium under more natural soil conditions are necessary. An experiment at present in progress indicates that decline of the fungus in infected straw is considerably slower under natural soil conditions outside than in the laboratory tumblers; this is to be attributed chiefly to lower soil temperatures, as loss of viability during the winter months is almost negligible.

In conclusion, reference may be made to the work of King *et al.* (1934), who have demonstrated the controlling effect of repeated applications of farmyard or green manure on the occurrence of cotton root rot, due to the fungus *Phymatotrichum omnivorum*, in Arizona. As a practical method for the control of this disease, King (1937) recommends the ploughing under of very heavy dressings of organic manures in deep furrows during autumn or early winter. One or more irrigations are given to encourage decomposition, which must also be favoured by the comparatively high soil temperatures (12–15° C.) of the Arizona winter season. In the spring, the rows of cotton are planted immediately over the buried decaying organic material. The striking reduction in the incidence of disease obtained by King on the manured plots may be attributed, in part at least, to a more rapid decomposition of the resting *Phymatotrichum* mycelium under such conditions.

V. Summary

A study has been made of the decline in viability of *Ophiobolus graminis* as resting mycelium in artificially infected wheat straw buried in the soil. The pieces of straw were buried in variously treated soils set up in glass tumblers, and were examined at intervals for the presence of still viable *Ophiobolus* mycelium by means of a wheat seedling test.

The experimental results suggest that the disappearance of *Ophiobolus* from the straws was due to natural decomposition by the other soil micro-organisms since, in its resting phase, the fungus tolerated adverse physical conditions of the soil better than conditions optimum for microbiological activity. Decline in viability of the fungus appeared to be indefinitely

postponed in air-dry soil, in soil at 2–3° C., and under sterile conditions in the culture flask; it was less rapid in a waterlogged soil than in one maintained at medium moisture content. The soil conditions least favourable for the advance of *Ophiobolus* along the host roots in its parasitic phase (Garrett, 1936) may best preserve it during its resting phase because they are also unfavourable for general microbiological activity.

Loss of viability was hastened by the addition of energy materials poor or entirely lacking in nitrogen, such as glucose, starch, and rye-grass meal, to the soil; it was more rapid in a partially sterilized and reinoculated soil than in an untreated soil. These results may be attributed to the rise in numbers and activity of soil micro-organisms following upon the treatments. The rate of decline of the fungus varied with soil type, being more rapid in rich and heavy soils than in poor, light soils. Rate of decline was apparently not directly affected by soil reaction, nor appreciably by moisture content of the soil over the range 30–80% saturation. Decline of the fungus was more rapid under conditions of fluctuating soil moisture and improved aeration in unglazed flower-pots than under more uniform conditions in glass tumblers; it was slowest in soil maintained under still and uniform conditions in a small closed incubator.

The most rapid disappearance of the *Ophiobolus* resting mycelium, therefore, seems to have been secured by conditions favouring maximum microbiological activity in the soil. The decline in viability of the fungus did not necessarily proceed parallel with gross decomposition of the infected straw as a whole. It was delayed by the addition of dried blood, containing 13% nitrogen, to the soil, whereas this treatment accelerated decomposition of the straw. Decline of the fungus was accelerated by addition of rye-grass meal, which delayed decomposition of the straw by taking up the available nitrogen. It is suggested that the *Ophiobolus* mycelium may itself serve as a source of nitrogen for the decomposition of the straw, and that the rapidity of its disappearance may be related directly to the degree of nitrogen scarcity in the soil and straw medium.

I have much pleasure in thanking Dr A. G. Norman for various useful suggestions, and for all the nitrogen determinations. I am especially indebted to Miss L. Cunow and to Miss M. M. Browne for help in the carrying out of the experiments.

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EXPLANATION OF PLATE XXVIII

Wheat seedling test for the presence of viable *Ophiobolus* mycelium in the infected straws. On left, four straws in which the viability of *Ophiobolus* is still undiminished; on right, four straws in which the fungus is no longer viable.

(*Received* 28 *March* 1938)

GARRETT.—Soil Conditions and the Take-all Disease of Wheat (pp. 742–766)

THE STERILIZATION OF LETTUCE SEED

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(With Plate XXIX and 7 Text-figures)

Introduction

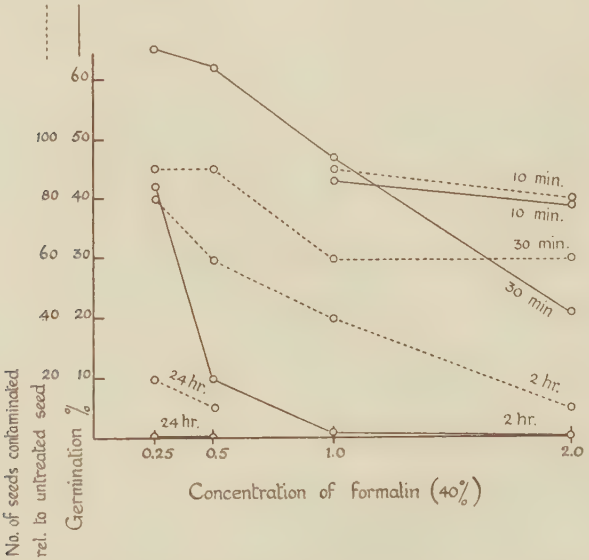
The marked development in recent years of methods of seed sterilization has been followed by attempts to control diseases—reputed to be seed borne—of lettuce seed. The application by lettuce growers of standard treatments recommended for cereals has resulted in serious damage to the seed. Similar cases of severe damage, quoted by Niethammer (1930), have been reported from Germany and Holland. Niethammer (1932) also records that lettuce seed fails to tolerate concentrations of organo-mercurial disinfectant that are innocuous to seeds of other vegetables, such as spinach and parsley. Moreover, disinfection by such a method was not efficacious in the case of lettuce seed. The comparative immunity of cereal seed to organo-mercurial chemicals has been attributed to the semi-permeable nature of the seed-coat, which absorbs relatively small amounts of mercury, whereas lettuce seed, which does not possess a semi-permeable coat, is said to absorb relatively large quantities of mercury. The present investigation was carried out in order to discover a safe and efficient method of sterilizing lettuce seed, which might be recommended to growers.

The effect of germicides at different concentrations and for different periods of treatment has been tested by (A) germinating treated seed upon a thin film of nutrient agar in sterile Petri dishes, the efficiency of the sterilization being estimated from the growth upon the agar of bacteria normally present on the seed coat, (B) sowing treated seed in sterilized soil in seed trays in order to ascertain the effect upon lettuce seed of treatments that have from time to time been recommended for the control of various seed-borne diseases.

A. Treated seed germinated in Petri dishes

The effects of various combinations of concentration of formalin and period of treatment on germination capacity and contamination of the seed-coat of the variety Golden Ball are given in Table I. These results

are represented graphically in Text-fig. 1, which shows that the slopes of the curves representing the fall in viability are steeper than those representing the fall in number of contaminated seeds. The effect of increase in potency of treatment is thus more marked upon seed injury than upon efficiency of sterilization. No treatment that allowed a high germination percentage gave satisfactory clean seed.



Text-fig. 1. The % germination of Golden Ball lettuce seed sterilized with different concentrations of formalin. The efficiency of sterilization, as estimated from relative number of seeds with viable bacteria, is also shown.

Table I. *The effect of concentration of formalin* (40 %) *and time of treatment on % germination of Golden Ball lettuce seed sown on nutrient agar. Values in brackets are relative numbers of seeds with viable bacteria on their coats. Germination of untreated seeds* = 58 %

	0·25 %	0·5 %	1·0 %	2·0 %
10 min.	—	—	43 (90)	39 (80)
30 min.	65 (90)	62 (90)	47 (60)	21 (60)
2 hr.	42 (80)	10 (60)	1 (40)	0 (10)
24 hr.	0 (20)	0 (10)	—	—

The effect on the viability of Golden Ball of all combinations of three concentrations of copper sulphate—0·5, 1·0 and 2·0 %—and three periods

of treatment—0·75, 2·0 and 4·5 hr.—is illustrated by the following results (mean of four samples with standard error):

1% for 0·75 hr.	1% for 4·5 hr.	2% for 4·5 hr.
46±3%	63±3%	33±4%

A concentration of 1% for 0·75 hr. does not affect viability (49% for untreated seeds), whereas the same concentration for a longer period increases the percentage of germinating seeds, possibly due to the suppression of contaminating organisms on the seed coat. Although the least potent treatment of 0·5% for 0·75 hr. reduced the number of contaminated seeds by 50%, about 10% of the seeds remained contaminated even after 2·0% for 4·5 hr., a treatment which has a definite detrimental effect on viability.

B. Treated seed sown in seed trays

Over 6000 seeds were sown in samples of about fifty. The following treatments were applied to each of the varieties Trocadero, Gotte à Forcer and Golden Ball:

Dip treatments		Dust treatments
Copper sulphate	1·0% for 1 hr.	Tillantin R
Mercuric chloride	0·25% for 15 min.	Semesan
Uspulun	0·25% for 1 hr.	Ceresan
Formalin	0·25% for 30 min.	Copper carbonate
Calcium hypochlorite (to give 2% Cl_2)	30 min.	Cuprous oxide

All the seeds treated by dips were washed in sterile water before sowing but a duplicate series (copper sulphate, mercuric chloride, and uspulun treatments) was carried out in which the seeds were dried before sowing without washing. The relative adhesiveness of the dusts was as follows: cuprous oxide = very good; Tillantin R, Semesan and Ceresan = satisfactory; copper carbonate = poor.

Table II. *The viability of lettuce seed sterilized by different methods and sown in seed trays. Treatments causing statistically significant decrease of germination are grouped below dividing lines*

Trocadero	%	Gotte à Forcer	%	Golden Ball	%
Untreated	82	Copper carbonate	79	Untreated	92
Cuprous oxide	82	Untreated	78	Copper carbonate	92
Copper sulphate	80	Copper sulphate	78	Uspulun	92
Semesan	79	Uspulun	77	Copper sulphate	90
Copper carbonate	78	Tillantin R	76	Formalin	89
Formalin	77	Cuprous oxide	75	Cuprous oxide	88
Uspulun	76	Calcium hypochlorite	72		
Calcium hypochlorite	75			Calcium hypochlorite	85
		Formalin	71	Semesan	75
Tillantin R	51	Semesan	67	Tillantin R	37
Mercuric chloride	2	Mercuric chloride	51	Mercuric chloride	1
Ceresan	0	Ceresan	8	Ceresan	1

The effect of the treatments on germination is shown in Table II, reductions that are statistically significant being separated by a line from those that are not. Injury less severe than that necessary to kill the seed is shown by retardation of germination, and many treatments that did not show final differences would have done so had an earlier count been considered. By plotting the daily stands the approximate extent of retardation may be estimated and is shown in Table III, the greatest number of untreated seeds germinating on the fourth day from sowing.

Table III. *Number of days retardation in germination of lettuce seed sown in seed trays following sterilization by different methods. The greatest number of untreated seeds germinated on the fourth day after sowing*

Trocadero		Gotte à Forcer		Golden Ball	
Cuprous oxide	5	Cuprous oxide	6	Cuprous oxide	4
Copper sulphate	5	Copper sulphate	0	Copper sulphate	5
Copper carbonate	0	Copper carbonate	0	Copper carbonate	0
Formalin	5	Formalin	5	Formalin	4
Uspulun	1	Uspulun	0	Uspulun	5
Calcium hypochlorite	0	Calcium hypochlorite	0	Calcium hypochlorite	0

From this experiment the following conclusions are drawn:

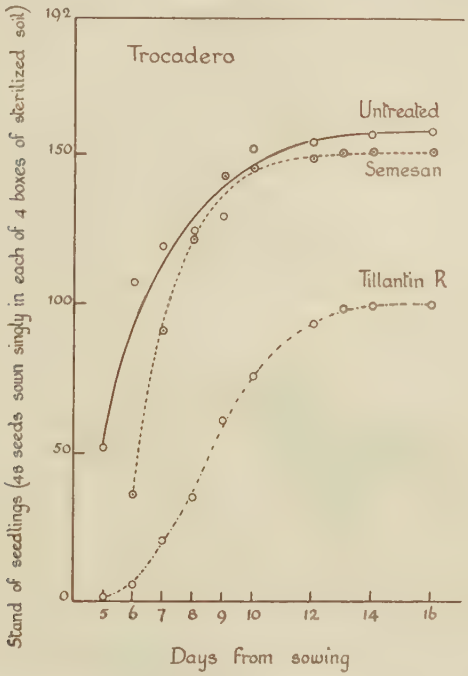
(1) With the exception of copper carbonate all the treatments were responsible on one variety or another for either a reduction in germination percentage or retardation of germination. The poor adhesiveness of copper carbonate suggests that its germicidal value is low. Since the concentrations of the dips used were weak (with the exception of mercuric chloride) it is evident that lettuce seed is abnormally sensitive and successful sterilization presents a more difficult problem than that of sterilizing cereal seed.

(2) Treatment with mercury was followed by severe seed injury. Mercuric chloride at the strength used was responsible for high mortality. Even where uspulun did not reduce the germination percentage an appreciable number of seedlings showed the deep green transparent cotyledons and blindness of the growing point symptomatic of mercury poisoning. The group of organo-mercurial dusts proved so toxic that their use for lettuce seed cannot be recommended.

(3) Appreciable reduction in the extent of retardation from copper sulphate and uspulun treatments followed the washing of the seed in sterile water before planting.

(4) The variety Gotte à Forcer is in general more resistant to seed injury than Trocadero or Golden Ball. 50% of Gotte seeds treated with

mercuric chloride germinated in comparison with 2% of Trocadero and 1% of Golden Ball. Copper sulphate and uspulun at strengths which severely retarded the germination of Golden Ball were innocuous to Gotte à Forcer. Resistance to seed injury in lettuce is a varietal characteristic.



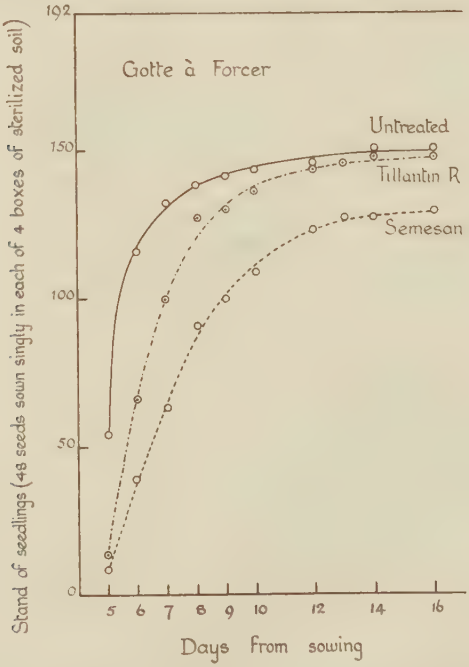
Text-fig. 2. Stand of seedlings (192 sown) of Trocadero, untreated and treated with Semesan and Tillantin R dusts (applied by shaking standard volume with measured quantity of seed in a mechanical shaker for standard period). The curves should be compared with those for Gotte à Forcer and Golden Ball in Text-figs. 3 and 4.

The differential effect of seed treatment on the germination capacity of lettuce varieties is illustrated in Text-figs. 2–4, which show the relative effects of Tillantin R and Semesan on the stands of Trocadero, Gotte à Forcer and Golden Ball respectively. The transition from the sigmoid curves characteristic of germination of injured samples and the decrement curves characteristic of the germination of untreated samples may be noted.

Trials with calcium hypochlorite

From the results of the preliminary trials calcium hypochlorite was selected as the most promising treatment on the following grounds:

(1) Only a small percentage—less than 7 %—of seeds so treated had failed to germinate in comparison with untreated seeds. (Subsequent

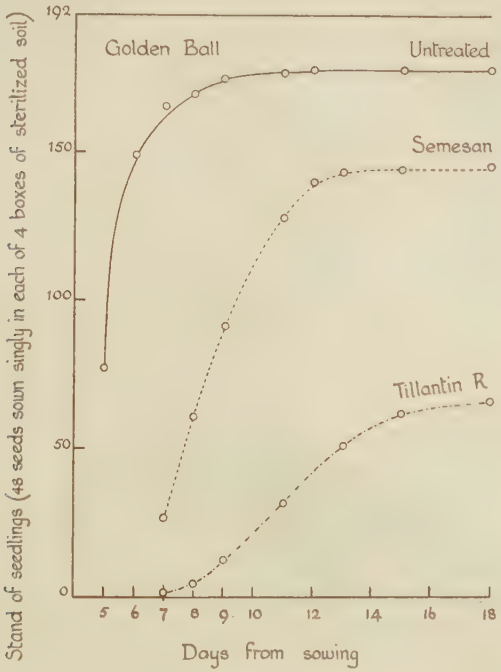


Text-fig. 3. Stand of seedlings (192 sown) of Gotte à Forcer, untreated and treated with Semesan and Tillantin R dusts (applied by shaking standard volume with measured quantity of seed in a mechanical shaker for standard period). The curves should be compared with those for Trocadero and Golden Ball in Text-figs. 2 and 4.

tests support the view that it was only the seeds already mechanically damaged that were affected.)

(2) The germination of treated seeds was not retarded and the seedlings all developed normally in contrast to treatments by mercury and copper preparations and formalin, which were followed by retarded germination and a percentage of abnormal seedlings clearly suffering from metal and other poisoning.

(3) A treatment that is partially gaseous is preferable in that irregularities in the seed coat may escape contact with sterilizing agents applied in the form of liquids and dusts.



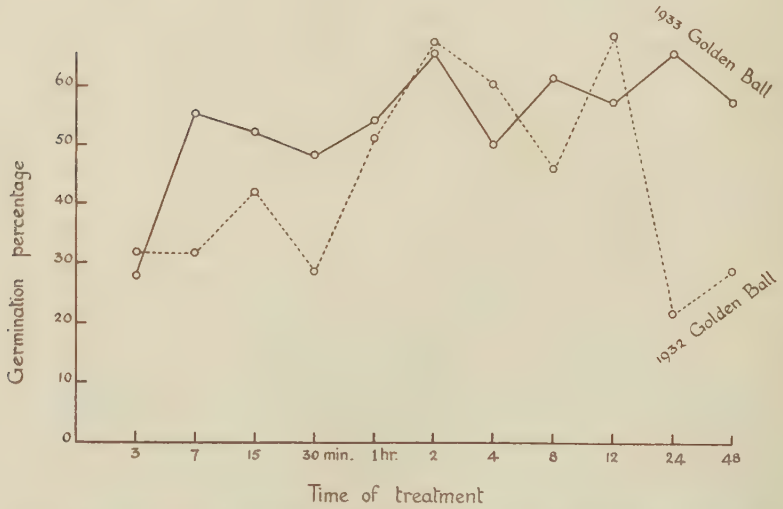
Text-fig. 4. Stand of seedlings (192 sown) of Golden Ball, untreated and treated with Semesan and Tillantin R dusts (applied by shaking standard volume with measured quantity of seed in a mechanical shaker for standard period). The curves should be compared with those for Trocadero and Gotte à Forcer in Text-figs. 2 and 3.

First set of trials, 29 *January* 1934

Triplicate samples of Golden Ball of about thirty seeds each were treated for periods ranging from 3 min. to 48 hr. and germinated in Petri dishes in contact with a thin layer of agar in order to show if the seeds were sterile. In addition to 1933 seed 1932 seed, that might be expected to possess less vigour, formed a parallel series. Table IV gives the summarized results. The germination capacity of 1932 and 1933 seed at different periods of treatment is plotted in Text-fig. 5.

Table IV. *The effect of duration of treatment with calcium hypochlorite on efficiency of sterilization and % germination of Golden Ball lettuce seed sown upon nutrient agar in Petri dishes*

Time of treatment	% germination		Number of Petri dishes (6 treated) in which all the seeds were sterile
	1932 seed	1933 seed	
3 min.	32	28	1
7 min.	32	55	1
15 min.	42	52	2
30 min.	29	48	3
1 hr.	51	54	4
2 hr.	67	65	6
4 hr.	60	50	3
8 hr.	46	61	4
12 hr.	68	57	4
24 hr.	22	65	4
48 hr.	29	57	4



Text-fig. 5. Germination percentage of 1932 and 1933 seed of Golden Ball (sown in Petri dishes on nutrient agar) plotted against time of sterilization with calcium hypochlorite.

The information provided by this set of trials is as follows:

(1) A sterile sample may be obtained in as short a time as 3 min. but 15 min. should be taken as the minimum time required to effect reliable sterilization (at 14° C. the temperature of treatment) since, after this period, 90% of the seeds treated were clean.

(2) An increase in the time of treatment from 3 min. to 12 hr. was associated with an increase in capacity of germination, independent of sterilization, since this was practically complete at all periods above 15 min. This rising trend of germination capacity may well be the result of an increase in supply of oxygen since, if seed is germinated on filter paper or in soil the level of germination capacity is appreciably higher (see Table V and also Text-fig. 7) and hypochlorite treatment has then little or no effect on viability. It is of interest to note that Rose (1915), Borthwick & Robbins (1928) and Thornton (1936) have demonstrated that the viability of lettuce seed may be increased by raising the level of oxygen supply.

(3) Germination was not impaired by any period of treatment save those for 24 and 48 hr. (1932 seed only). Thus, even if the maximum period tolerated is only 12 hr., ample margin of safety is provided by the method since reliable sterilization was affected in as short a time as 15 min.

Second set of trials, 12 *February* 1934

Triplicate samples of Golden Ball (1932 and 1933 seed) were treated for periods ranging from 15 min. to 96 hr. As the previous trial had shown sterilization to be satisfactory after 15 min., and it was considered possible that the agar might interfere with germination, the seed was germinated upon moist filter paper. Very good germination was obtained, that of the untreated samples being 89% (1933 seed) and 90% (1932 seed). Thus the final germination capacity of 1932 and 1933 seed does not differ but the greater vigour of the 1933 seed was shown by earlier germination, counts at the end of 24 hr. giving the following values:

1932 seed 43 ± 4% germination. 1933 seed 55 ± 2% germination.

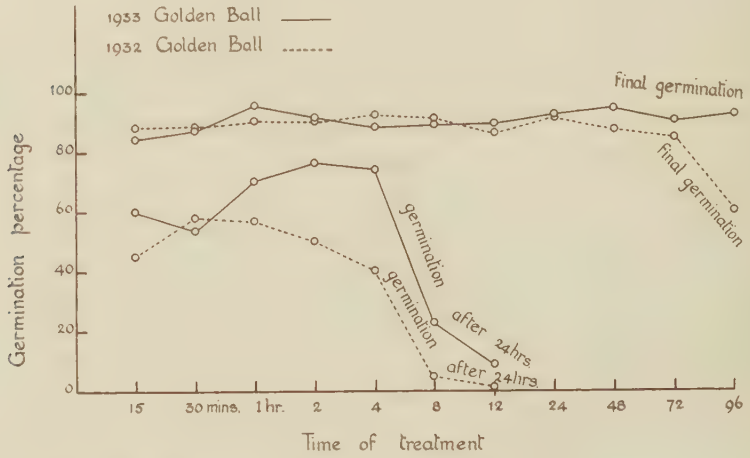
Table V. *The effect of duration of treatment with calcium hypochlorite on the germination capacity of Golden Ball lettuce seed sown upon moist filter paper in Petri dishes*

Time of treatment	1st day's germination %		Final germination %	
	1932 seed	1933 seed	1932 seed	1933 seed
15 min.	45	60	88	84
30 min.	58	54	88	87
1 hr.	57	70	90	95
2 hr.	50	76	90	91
4 hr.	40	74	92	88
8 hr.	5	23	91	89
12 hr.	1	9	86	89
24 hr.	—	—	91	92
48 hr.	—	—	87	94
72 hr.	—	—	84	90
96 hr.	—	—	60	92

The effect of the treatments on germination capacity are shown in Table V and Text-fig. 6, from which the following conclusions are drawn:

(1) The final germination percentage of 1933 seed has been unaffected by treatments for as long a period as 4 days, that of 1932 seed, of less vigour, for 1 or possibly 2 days.

(2) A retarding effect upon germination is apparent for periods above 4 hr. (1933 seed) and 1 hr. (1932 seed).



Text-fig. 6. Germination % of 1932 and 1933 seed of Golden Ball (sown in Petri dishes on moist filter paper) plotted against time of sterilization with calcium hypochlorite.

With a weaker solution of hypochlorite (allowed to stand for 4 hr. before use) germination was not retarded after periods of treatment less than 48 hr. (1933 seed) and was even accelerated, for the initial days germination after treatment for this period was 84% (88, 83, 82) for treated seed in comparison with 55% (57, 51, 58) for untreated seed. In contrast the germination of the 1932 seed was retarded after treatment for the same period, the values for the initial days germination being 23% (38, 20, 11) against 43% (51, 43, 36) for untreated seed.

Sterilization clearly has a differential effect upon seed samples of the same varietal constitution but differing in vigour. Treatments that leave unharmed seed of strong constitution may be unsafe to apply to older seed of less vigour.

Third set of trials, 11 *April* 1934

In these trials 1933 seed treated for periods ranging from 3 min. to 72 hr. was divided into two parallel series, one being germinated on filter paper and the other sown in the usual commercial manner in trays. In order to eliminate any possible differential effect of soaking prior to sowing the unsterilized seed, samples were soaked in glass-distilled water for periods corresponding with those of the sterilization treatment. The results are shown in Table VI.

Table VI. *The effect of duration of treatment with calcium hypochlorite on the % germination of Golden Ball lettuce seed sown on moist filter paper in Petri dishes and in sterilized soil in seed trays*

	Seed germinated in Petri dishes				Seed sown in trays			
	Examination after 1 day		Final examination		Examination after 4 days		Final examination	
Time of treatment	Sterilized	Soaked	Sterilized	Soaked	Sterilized	Soaked	Sterilized	Soaked
3 min.	49	49	85	91	39	30	84	85
7 min.	58	47	85	95	47	34	91	77
15 min.	51	48	87	88	44	38	89	71
30 min.	55	47	87	86	20	26	85	83
1 hr.	46	42	85	87	25	36	86	86
2 hr.	47	45	89	92	31	27	96	78
4 hr.	48	27	81	81	33	31	96	80
8 hr.	9	7	90	84	29	19	89	90
12 hr.	—	—	87	90	31	6	96	88
24 hr.	—	—	90	91	—	—	93	85
48 hr.	—	—	83	90	—	—	78	89
72 hr.		—	77	77	—	—	76	96

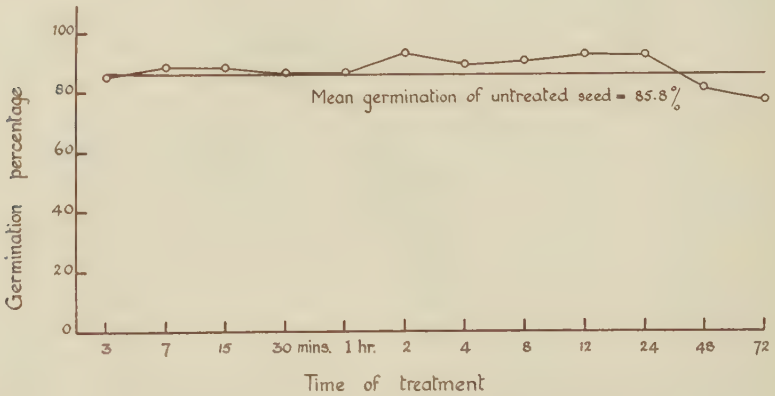
Seed germinated in Petri dishes

The mean germination percentage of the sterilized samples 86·3% compares favourably with the mean 88·6% of the soaked samples, and demonstrates that the treatment is harmless though applied for periods many times as long as that necessary for efficient sterilization. The values in Table VI for germination capacity suggest a tendency for earlier germination of the treated seed.

Seed sown in seed trays

The relation between the sterilized and untreated but soaked samples remained the same as in Petri dishes. For seven out of nine periods of treatment the percentage germination of the sterilized seed was higher, 4 days after sowing, than that of the untreated but soaked seed. This stimulating effect of hypochlorite treatment cannot be correlated with

germicidal action, which is presumably fully exerted after the shorter periods of treatment but is probably attributable, as in the case of viability, to increase of oxygen supply. The final percentage germination of seed sown in trays by a similar method to that used by growers was not reduced below that of the untreated seed save after long periods of treatment of 48 and 72 hr. The effect on germination percentage of period of sterilization may be seen in Text-fig. 7 in which are combined the results obtained by sowing on filter paper in Petri dishes and in soil in seed trays.



Text-fig. 7. Germination % of Golden Ball (sown in Petri dishes and in seed trays) plotted against time of sterilization with calcium hypochlorite. The mean germination percentage of samples unsterilized but soaked in water prior to sowing is 85·8.

Method recommended for the sterilization by growers of lettuce seed

The method used, believed to have been originally introduced by Wilson (1915), consists of adding calcium hypochlorite ("bleaching powder") to distilled water at the rate of 5 g. per 70 c.c. (or roughly $\frac{3}{4}$ lb. per gal.), stirring, leaving for 5 min. to settle, decanting and using immediately. The original volume should be calculated so as to allow of the rejection of a third of the liquid during decantation, sufficient being left to just cover the seed. The latter may be placed in a glass bottle, which can be corked and shaken periodically to overcome the tendency of the seed to float on the surface. 4–8 hr. is recommended as a suitable period but any period between 15 min. and 24 hr. should give satisfactory results. Below 15 min. sterilization may be incomplete and above 24 hr. there is risk of seed injury. Washing the seed after treatment reduces the

risk of seed injury. Preparations of bleaching powder differ markedly in their chemical activity and the sterilization of large quantities of seed should be prefaced by observation of its effect on a small sample, which may be germinated on moist blotting paper.

Summary

1. Since severe damage has followed the application by growers to lettuce seed of standard methods of sterilization recommended for cereals, trials of different methods of sterilizing lettuce seed have been carried out. Satisfactory sterilization free from seed injury is not effected by copper sulphate or formalin, while preparations containing mercury such as organo-mercurial dusts are highly toxic to lettuce seed. Sterilization with calcium hypochlorite is a safe and efficient method.

2. Treatment with calcium hypochlorite prior to sowing has an accelerating effect, independent of germicidal action, upon the germination of lettuce seed. The viability of seed sown on filter paper or in soil is not appreciably affected but that of seed sown in contact with a film of agar, which shows exceptionally poor capacity to germinate, is notably increased. These effects are not attributable to germicidal activity and possibly result from increase of oxygen supply.

3. Susceptibility of lettuce seed to injury from germicides is a varietal characteristic.

4. Fresh seed with strong viability is more resistant to injury from germicides than older seed of less vigour.

Grateful acknowledgement is due to Dr G. C. Ainsworth for taking the photographs in Plate XXIX and for reading and criticizing the MSS.

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EXPLANATION OF PLATE XXIX

Fig. 1. The toxic effect of an organo-mercurial disinfectant upon lettuce seed. The seeds sown in the box "*a*" were treated prior to sowing with cuprous oxide dust, whereas those in boxes "*b*" and "*c*" were treated with a proprietary organo-mercurial dust.

Fig. 2. Varietal resistance to injury from seed disinfectant. The seedlings in box "*a*" are the relatively resistant variety Gotte à Forcer and, in boxes "*b*" and "*c*" respectively, the relatively susceptible varieties Trocadero and Golden Ball. All seeds have been dipped prior to sowing in the same concentration of mercuric chloride for the same period.

(*Received* 21 *March* 1938)

Fig. 1.

Fig. 2.

WHITE.—The Sterilization of Lettuce Seed (pp. 767–780).

VEIN CLEARING AND VEIN BANDING INDUCED BY HYOSCYAMUS III DISEASE

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(With Plate XXX and 3 Text-figures)

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Introduction

A clearing or yellowing of those areas of the leaf immediately aligning the veins is a frequent sign of virus infection. With many viruses and in many hosts it is the first symptom to become visible macroscopically. A virus may produce this symptom almost invariably in some hosts but never in others. It can sometimes be encouraged or precluded by varying the conditions of growth, as for instance in curly top of sugar beet (Severin, 1929) where transparent venation occurs under glasshouse conditions. The symptom is usually short lived. In tomato infected with aucuba mosaic disease (Henderson Smith, 1928) it is soon replaced by a mottle. In tobacco infected with tobacco streak virus (Johnson, 1936) vein clearing is followed by necrosis. This paper is concerned with tobacco infected with Hyoscyamus III disease (Hamilton, 1932). In this case, vein clearing (Pl. XXX, fig. 1) may be followed later by vein banding (Pl. XXX, fig. 2). A few days after inoculation a strip up to 1 mm. in width along each side of each of the principal veins becomes yellow. The cleared condition persists for about 2 days. Then the yellow regions become diffuse and, after a further week or so, dark green bands appear along the whole or part of the length of many of the veins. Those areas

where banding occurs were thus previously devoid of colour. The bands vary in width from very narrow to several millimetres.

What are the anatomical or cytological changes which result in these macroscopic differences? Clearing might be brought about by a proliferation of the vascular strand or by inhibition or retardation of the development of the plastid primordia, of the plastids or the pigment itself. As clearing is followed by banding any destructive process would need to be followed by a process of regeneration.

Methods

For microchemical tests and certain types of examination, hand sections, frozen sections and whole mounts were used. Hand and frozen sections, unless for microchemical tests, were mounted in isotonic salt or sugar solution. For most of the study, material was fixed, embedded and sectioned by the microtome.

Young tobacco plants were inoculated with Hy. III disease. Immediately vein clearing became visible macroscopically in *one* leaf, that leaf and all the younger ones were cut up and fixed. For vein banding, leaves showing this symptom were cut into small portions each containing a vein surrounded by dark green tissue at the margin of which a small amount of the yellower tissue was left. These pieces were fixed immediately.

For general fixation, Zenker's fluid was best at all stages. For cytological details, such as plastid development, Champy's fluid gave the best results. Some material was dehydrated, cleared in cedar-wood oil, embedded in paraffin wax and sectioned in the usual way at 6–15μ. Whole mounts were made by staining the fixed material in bulk, clearing in dioxan and mounting in dioxan balsam. The stains used chiefly for the sections were crystal violet and erythrosin, safranin-light green or Feulgen's reagent with light green or orange G after Zenker fixation. After Champy's fluid, Heidenhain's haematoxylin or aniline-fuchsin-picric acid were employed. Fixed material to be mounted whole was stained in Feulgen's reagent and counter-stained with orange G, light green or picric acid.

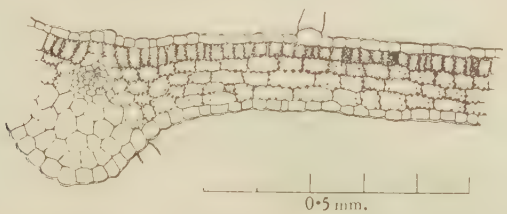
Description

As a preliminary both vein-cleared and vein-banded leaves were tested for the presence of starch. Leaves were taken from plants in the glass-houses at 4 p.m., dipped in boiling water, decolorized in alcohol and stained in iodine solution. Leaves so treated presented a striking picture.

In the younger series, a meshwork of unstained tissue contrasted with the blue colour of the interveinal areas. In the older series, dense black areas closely aligning the veins stood out against the now paler blue interveinal regions. The absence of starch from the cleared parts, its abundance in the banded parts, and its relative scantiness in the yellowish regions of the banded leaves, indicate the abnormal colouring of the leaves to be due to abnormal development or distribution of the chloroplasts and not to any masking effect.

Anatomy of the leaves

The anatomy of the leaves was examined, special attention being paid to the vascular strand. Phloem is visibly affected by many viruses. Phloem necrosis occurs in the potato (Quanjer, 1913), and "transparent venation" in sugar beet may be correlated with hypoplasia in the phloem (Esau, 1933). As in plants infected with Hy. III disease the clearing is

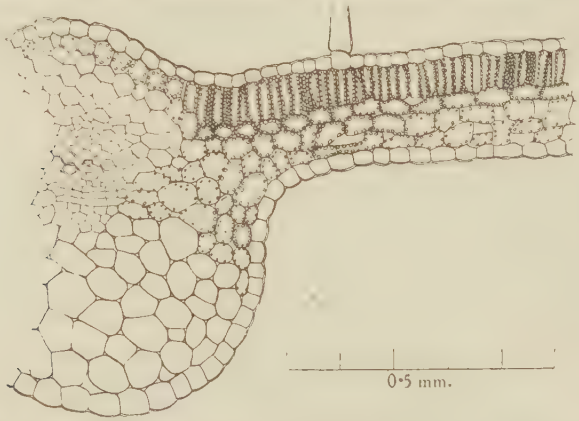


Text-fig. 1. Transverse section of infected leaf showing vein clearing.

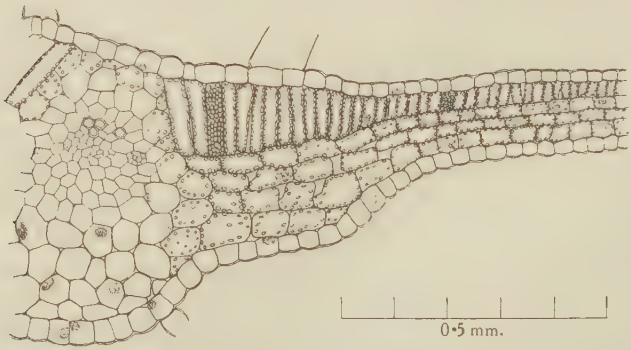
confined to those areas immediately approximating the veins, it seemed possible that some abnormality might occur in the phloem. However, an exhaustive examination of the development and structure of the vascular strand threw no light on the origin of the cleared areas. In the earlier stages abnormalities were apparent neither in the vascular strand nor in the general anatomy of the leaf (Text-fig. 1). The smaller vascular strands each consist of three or four tracheids and a few phloem cells and are completely surrounded by spherical parenchymatous cells. The assimilatory tissue is not at this stage fully developed and consists of closely packed cells usually roughly cubical or cuboidal in shape. At the time vein clearing becomes apparent these contain many plastids. The latter are present in approximately equal numbers in the cleared and in the green areas.

When vein clearing becomes apparent almost all cell division has ceased, but the cells are still growing in size. By the time vein banding is apparent, it is obvious that the leaf development has for the most part

been retarded although in certain cells growth has been excessive. Banded leaves are stunted when compared with corresponding ones of normal plants. Anatomically the vascular strand is still normal but the structure of the interveinal areas is now quite abnormal (Text-fig. 3). The banded areas are much thicker than the surrounding yellow parts



Text-fig. 2. Transverse section of healthy leaf of same age as lea in Text-fig. 3.



Text-fig. 3. Transverse section of infected leaf through a very narrowly banded vein.

of the leaf, the palisade cells in the former being more elongated than those in the normal leaf (Text-fig. 2), whilst, in the latter, very little elongation has occurred.

The hyperplasia of those palisade cells aligning the vein occurs at the expense of the spherical parenchymatous cells above the vein. These are fewer in number than in a healthy leaf. This excessive growth also causes

considerable widening of the bulge which normally occurs over the vascular bundle. Below the much-elongated palisade cells are found unusually large parenchymatous cells with well-developed air spaces. In the yellower regions, the cells of the spongy parenchyma have developed but little since clearing occurred around the veins and intercellular spaces are much smaller than in the normal leaf. The differences in colour in the different parts of the leaf are now due to differences in the size of the cells and, possibly, to the amount of chlorophyll which can be accommodated in a cell of a given size.

Cytology of the leaves

On examining the cells in detail one abnormality immediately becomes apparent, not in the cleared leaves but very conspicuously in the banded ones. Intracellular inclusions occur in abundance in almost all tissues of the older diseased leaves. They are not found in the xylem but occur in large numbers in the phloem, in the epidermis, and in all types of parenchymatous tissue. (Text-fig. 3).

The anatomical examination having thrown but little light on the problem of the distribution of the chlorophyll, on examining the individual cells, special attention was paid to the development and structure of the plastids. It has been found that the yellow mottle in certain plants infected with yellow mosaic disease of tomato (Sheffield, 1933) is produced through inhibition of the usual processes of development of the chloroplasts from the proplastids. It was thought that vein clearing might be due to some analogous cause. However, so far as can be determined visually, the plastids of plants infected with Hy. III disease develop from the primordia in the normal way. The proplastids, at first minute bodies indistinguishable from the chondriosomes, become vacuolate and increase in size until ultimately mature plastids are formed. These are lenticular in shape and consist of an almost colourless stroma in which are embedded minute coloured lenticular particles which presumably contain the pigments. Plastids from the cleared areas give the impression of being yellowish in colour rather than green, as are those from the normal areas. The minute lenticular granules can be seen dispersed throughout the plastids but they are too small for it to be possible to determine whether they are of their usual depth of colour.

Plastids are present in cleared areas and in the corresponding position in healthy leaves in approximately equal numbers. There is, however, some variation in size. Plastids vary in size in any one leaf, especially according to their position. Slight variation occurs within a single cell.

In a healthy leaf plastids from the spherical parenchymatous cells near the veins are usually about 3μ in their largest diameter. In the palisade cells the longest diameter of the plastids may reach as much as 6–7μ. In the cleared areas of the infected leaves the plastids over the vascular strand may be only 2μ in diameter, and they are seldom more than 4μ in the palisade cells. A few days later, when "banding" has replaced "clearing", the average long diameter of the plastids in these same cells is often greater than that of the plastids in the healthy leaf.

Pigmentation of the plastids

It is evident from the occurrence of so little structural abnormality to account for the yellowing of the leaf areas near the veins subsequent to infection, that the inhibiting influence comes into action at a later stage than in the case of aucuba-infected plants. It suggests that the development of the pigment may be in some way abnormal. Cleared areas were therefore dissected under the microscope from infected leaves and similar portions were taken from healthy leaves. The chlorophyllan test was carried out on both. On treatment with glacial acetic acid, clusters of straight needle-like crystals were produced by the healthy tissue. The crystals were soluble in hot glacial acetic acid, chloroform, ether, or alcohol and insoluble in potassium hydroxide. From diseased tissue similar crystals were obtained but in very small numbers. Their production by diseased cells may be due to the presence of small amounts of pigment in the diseased areas but is more likely due to the difficulty of completely isolating a "cleared" area from the attached green cells. Tests for carotin and xanthophyll were also carried out but were far from satisfactory, as very small amounts were obtained even from healthy plants. Material, dissected as before, was put into chloroform; after a few minutes either petrol ether or methyl alcohol was added. The former resulted in the separation of xanthophyll and the latter in the separation of carotin. No differences were observed in the amounts of these pigments obtained from different sources.

Distribution of the virus

The microscopic examination suggested that the action of the virus is first to delay the production of chlorophyll in those parts of the leaves which it first reaches. It then spreads from the veins across the interveinal areas. The areas near the veins are now able to develop further, whilst cell development is retarded in the interveinal regions. It seemed possible that the virus is at first present around the veins in great concentration and then, as "clearing" gives place to "banding", the virus

not only spreads from this region but is actually withdrawn from it. Experiments were designed to test this hypothesis.

Exp. I. The virus content of cleared areas of young leaves was compared with that of the banded regions of older leaves. Strips were dissected from the leaves: it was impossible to measure these accurately, but all were of approximately the same area. From the "cleared" parts it was possible to cut strips only about 0·5 mm. in width if the tissue of the vein was to be excluded. Strips were taken 1 mm. in length. To calculate the volume, the leaf was assumed to be 0·25 mm. in thickness. The volume of each piece was thus $(0{\cdot}25 \times 0{\cdot}5 \times 1 \times 10^{-3})$ c.c. or (125×10^{-6}) c.c. Actually, the thickness of the banded areas is about twice as great as that of the cleared, so the volume was also twice as great and also the resulting concentration in the extract. Such a variation was immaterial as compared with the type of difference which was expected, especially as no accurate measure was available for any of the figures. 100 pieces of each type of tissue were taken. Each series was crushed, extracted with 1·25 c.c. water, giving a dilution of approximately 1/100, and inoculated into one leaf of each of six tobacco plants. After an appropriate interval the leaves were removed from the plants, killed and starch lesions tested for. The plants were kept for systemic infections. This experiment was repeated twice with the following results:

		Total nos. of lesions	
Cleared tissue. Exp. I*a*	320	553	
,, ,, ,, I*b*	233		
Banded ,, ,, I*a*	98	182	
,, ,, ,, I*b*	84		

All plants showed systemic infection.

Exp. II. The virus content of the banded areas was compared with that of the yellow regions between the bands. 300 pieces 1 × 1 mm. were dissected from each type of tissue. The volume of each piece was approximately $(1 \times 1 \times 0{\cdot}25 \times 10^{-3})$ c.c. or 25×10^{-5} c.c. Each series was crushed and extracted with 3 c.c. of water giving a dilution of 1/40. Each series was inoculated into one leaf on each of fifteen different plants. The green tissue gave 587 lesions against 911 from the yellow tissue. All plants showed systemic infection.

Exp. III. Tissue from "cleared", "banded" and yellow areas was compared. 200 pieces each $(1 \times 1 \times 0{\cdot}25 \times 10^{-3})$ c.c. were cut from banded and yellow areas and 400 pieces $(1 \times 0{\cdot}5 \times 0{\cdot}25 \times 10^{-3})$ c.c. from cleared parts. Each series was crushed and extracted with 5 c.c. water to give a dilution of 1/100. The three series were inoculated into randomized half tobacco leaves. The following numbers of lesions resulted:

Cleared tissue	1644
Banded ,,	553
Yellow ,,	1224

In Exps. I and III the numbers of lesions obtained from the cleared areas was regularly about three times as great as the number obtained from the banded parts, indicating a somewhat higher concentration of virus in cleared tissue than in banded. Exps. II and III show the yellow interveinal areas of older leaves to contain more virus than the banded parts and less than the cleared areas. When these experiments were designed it was expected either that very considerable differences would

be found if the clearing were due to a direct effect of the virus on the pigment or, if the initial hypothesis were untrue, then no appreciable differences were expected. To support the hypothesis differences would need to be of a greater order of size than those shown.

The differences found are, because of their constancy, not without interest and, actually, are slightly greater than the figures indicate. As large differences were expected, it was assumed in designing the experiments that all leaves were of the same thickness. Text-figs. 1 and 3 indicate the banded parts to be about twice as thick as the cleared or yellow parts, so that a dilution of 1 100 of cleared and yellow tissues was actually compared to a concentration of 1/50 of banded tissue. From unpublished data of Dr M. A. Watson, it has been estimated[1] that at the appropriate part of the curve (i.e. where about thirty lesions occur per leaf) a drop in concentration to 1/10 results in a fall in the number of lesions to 1/5–1/10. It can thence be calculated that the virus content per unit volume of cleared tissue is probably 6–11 times as great as that of banded tissue. It is possible that the virus produced around the veins may, subsequently, be withdrawn, but it seems more probable that differences in concentration are due to variations in cell size, the concentration of the virus being a function of the number of cells in a given volume of tissue. The reduction in concentration is of the right order of size to be attributable to this cause. Some micrurgical studies have suggested that a large amount of virus is produced in the cell in a short period immediately following the time infection reaches it (Sheffield, 1938). The virus presumably first reaches those cells approximating the veins and these rapidly produce large amounts of virus. The infection passes out from these cells into the interveinal areas, each cell of which may produce virus approximating in quantity to that produced by each cell near the vein. Differences in concentration are then brought about by unequal increase in cell size. Hyperplasia occurs in those cells near the vein and, consequently, the virus concentration is there greatly reduced but, in the interveinal areas, little increase in cell size seems to occur after entry of the virus. Consequently, the concentration in these cells is not much less than in the cleared parts.

Why the virus should first inhibit and later stimulate the production of pigment in some cells and retard it in other cells, and why hypertrophy should occur in some tissues and inhibited development in others, cannot yet be explained. It might be suggested that the stimulation which is often evident in infection (Sheffield, 1931, 1936) here causes hyperplasia

[1] By Mr W. G. Cochran of the Statistical Department.

Fig. 1.

Fig. 2.

SHEFFIELD.—Vein Clearing and Vein Banding induced by Hyoscyamus III Disease (pp. 781–789).

in the cells of the banded tissue, but it is difficult to understand why the same agent should inhibit growth in other cells.

Summary

The first symptom of Hy. III disease in tobacco is a clearing of the veins. This is followed later by vein banding. During clearing no anatomical or cytological abnormalities occur. The yellow colour is due to a retardation of chlorophyll formation.

When vein banding becomes apparent considerable hypertrophy is seen in the tissues near the veins and hypoplasia is apparent in the interveinal areas. Intracellular inclusions are abundant in all tissues except the xylem.

Cleared tissue contains 6–11 times as much virus per unit volume as does the banded tissue. The latter also contains less than do the yellower parts of banded leaves.

I am indebted to Fraulein Lina Cunow who made most of the permanent preparations used in this study.

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EXPLANATION OF PLATE XXX

Fig. 1. Leaf of tobacco infected with Hyoscyamus III disease, showing clearing of veins (control leaf above).

Fig. 2. Leaf of tobacco infected with Hyoscyamus III disease, showing vein banding. (Reduced by $\frac{1}{5}$).

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STUDIES OF THE MOSAIC DISEASES OF CASSAVA

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(With Plates XXXI and XXXII and 1 Text-figure)

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Introduction

A mosaic disease of cassava (*Manihot utilissima* Pohl), first reported in East Africa under the name of "Kräuselkrankheit" by Warburg (1894) and studied by Zimmermann (1906), is now prevalent in East Africa and the adjacent islands. More recently, it has been reported from most countries of western and central tropical Africa (Dade, 1930; Deighton, 1932; Dufrénoy & Hédin, 1929; McKinney, 1929; Pascalet, 1932; Staner, 1931), from Madagascar (Bouriquet, 1932; François, 1937), and from Java (Muller, 1931). There has been growing recognition of the serious losses that the disease entails.

That this disease is caused by a virus was suggested by Zimmermann (1906), and this is now accepted by most workers, although some have sought to attribute it to a visible parasite (Kufferath & Ghesquière, 1932; Strong & Shattuck, 1930).

For general accounts of the disease in the field the papers listed under "References" may be consulted (particularly Pascalet, 1932; Staner, 1931; Bouriquet, 1932). In this paper we describe studies carried out at the East African Agricultural Research Station, Amani, mainly upon the strains of the mosaic virus and their transmission.

Since 1935 we have recognized a second disease, known as "brown streak", believed also to be caused by a virus, but one distinct from the mosaic group. A brief account of our present knowledge of this disease has been published (Storey, 1936).

Symptoms and their variations

The symptoms in cassava are characteristic of a mosaic disease—primarily chlorosis of discrete areas of the leaf-lamina. The chlorotic areas are determined during the early development of the leaf, and these areas fail to expand fully, so that stresses set up by unequal enlargement of adjacent areas cause distortion of the leaflets. The typical picture is a leaf, reduced in size, misshapen and twisted, with bright yellow areas separated by areas normally green (Pl. XXXI, fig. 1).

Great variations may be seen in the symptom manifestation, both between different plants and between different leaves of a single plant. We may analyse the symptoms as follows:

(*a*) *Chlorosis.* The chlorotic tissue may be a pale yellow or nearly white, with only a tinge of green; or, at the other extreme, it may be only just discernibly paler than normal.

(*b*) *Size of the chlorotic areas.* A "chlorotic area", although containing small "islands" of green tissue, particularly along the veins, is usually well demarcated from the green areas. It may vary in size from that of the whole leaflet (Pl. XXXII, fig. 4) to small flecks or spots (Pl. XXXII, fig. 3).

(*c*) *Frequency of the chlorotic areas.* All leaflets may show a nearly uniform mosaic-pattern (Pl. XXXI, figs. 1 and 2); or the mosaic-pattern may be localized in a few areas only (Pl. XXXII, fig. 4). Often chlorosis is confined to the base of the leaflet (Pl. XXXI, fig. 3); this is found, not only during early development following infection, when it would be well understood, but also in plants long systemically infected.

(*d*) *Distortion.*

(*e*) *Reduction in size of leaflets.*

(*f*) *General stunting.*

These last three characters all appear to be secondary, in that their extent in the leaf, or in the plant as a whole, can be related to the severity

of the symptoms under (*a*) to (*c*). Thus a severely chlorotic area grows less than one mildly chlorotic, so that the leaflet is smaller and more distorted. A plant with only mild chlorosis, or with severe chlorosis affecting only a small proportion of its total leaf area, makes stronger growth than one severely affected.

To relate the observed variations in symptoms to their causes has proved a difficult problem. One reason is the existence of more than one strain of the virus: the differences in the symptoms characteristic of the virus strains will be considered in the next section. Another reason is to be found in the different degrees of tolerance inherent in the cassava varieties. Although at present we have little exact evidence on varietal response, we can quote one striking example: the virus producing in an arborescent cassava variety only a mild localized mosaic, on transfer by grafting to the Mbarika variety, produced a typical severe mosaic.

However, within plants of a single clone, carrying a single virus strain (or conceivably mixture of strains) differences in symptoms occur from time to time or from place to place. Even in the successive leaves of a single shoot differences may often be seen as great as those of the shoot illustrated in Pl. XXXI, fig. 3, which, between stunted leaves predominantly chlorotic, bore one leaf about half chlorotic and one normal in size and colour except for a few chlorotic flecks. This kind of effect may sometimes give the appearance of complete recovery. Field experience suggests that true recovery may occur in nature, as Deighton has reported (1935); but none of the plants that we have studied has failed to revert to the diseased condition. Differences in the severity of the chlorosis may be observed, particularly with the "mild" virus strains (see next section). A single mild strain in a single clone may sometimes produce relatively severe symptoms (Pl. XXXII, fig. 2), sometimes only a faint paleing of the normal green (Pl. XXXI, fig. 2), and at yet other times no chlorosis whatever.

In part, symptom expression is determined by the age of the affected shoot. The first few leaves produced by a cutting carrying the virus are often symptomless. Thereafter the severest symptoms characteristic of the particular strain usually appear; but then as the plant grows there is often a tendency for the severity to diminish. If the shoot be cut back, severe symptoms generally reappear in the young growth from axillary buds. When newly infected a plant tends to pass through an early severe phase from which later it partly recovers.

The environment as a whole can greatly influence the symptom expression in a single clone affected by one virus strain or mixture of

strains. It is uncertain how far nutrition, water supply and illumination may have contributed to the differences that we have observed. Probably, temperature is the most important controlling factor and, in general, the lower the temperature the more severe the manifestation. There is an indication, however, that temperature may not affect all the virus strains similarly.

Virus strains

The large influence that external factors may exert on symptom manifestation shows the need for caution in any attempt to differentiate strains of the mosaic virus on a basis of symptoms alone. We have, however, at present no other means of differentiation. The best that can be done is to study the symptoms in plants of a single clone, all grown under identical conditions.

Early in 1935 we collected in the field near Amani forty cuttings from diseased plants showing as wide a range of symptoms as could be found. After preliminary study, twenty-two of these were grafted on to plants of a healthy clone of the variety Mbarika. Duplicate cuttings of all the Mbarika plants, so infected, were planted on the same day in the greenhouse; and at intervals of a few months further cuttings were taken and planted. The symptoms in these plants were observed through 15 months. From time to time a single plant might exhibit considerable variation in its symptoms but, at any one time, the symptoms in the duplicate plants of similar age and having a single origin were closely similar.

These studies have enabled us to divide the material into two groups. Since the only known variable in the material was the causative virus, we have made a corresponding division into two groups of virus strains. The characteristic symptoms produced by each, under the special environmental conditions, are given below.

Conditions of study. Nutrition apparently optimal. In glasshouse, at lat. 5° S. and 3000 ft. altitude; overhead slatted shade obstructing 50% of sky, during hours of sunshine. Temperature uncontrolled: approximately as follows:

Annual mean: 24° C.

Hottest month: mean of daily maxima, 32° C.; mean daily range, 11·5° C.

Coldest month: mean of daily minima, 17° C.; mean daily range, 8° C.

Severe group. Chlorosis severe, yellow or sometimes nearly white; chlorotic areas usually large, usually more or less uniformly distributed,

but sometimes localized. Type G 1 (Pl. XXXI, fig. 1). Other severe types have conformed fairly closely to G 1, except that some have shown a greater tendency for the pattern to be localized to small areas of the leaf.

Mild group. Chlorosis slight, affected areas only slightly paler than normal; chlorotic areas small, either generally distributed or localized. Often symptomless. Type D 2 (Pl. XXXI, fig. 2). Other types, although included in this group, showed some differences from D 2. Thus D 11, although showing the same kind of symptoms (Pl. XXXII, fig. 1), was usually more severe than the D 2 growing alongside; but occasionally it was less severe. It was less frequently symptomless than D 2. G 21 only rarely produced faint flecks and was usually symptomless. D 7 and D 8 were always symptomless in the greenhouse, and were doubtfully placed in this group on the evidence of indefinite symptoms shown in the field. [The difference between D 2 and D 11 was more marked when these plants were grown in the open at Amani, in a valley subject to cold-air drainage; here D 2 showed a moderately severe greenish mosaic (Pl. XXXII, fig. 2); while at the same time D 11 showed only faint flecks or was symptomless.]

The difference between the symptoms produced by the severe and mild strains is so great that the validity of separating them into the two groups can hardly be questioned, even though some of the material studied may have contained a mixture of strains. So long as we lack any means of analysing a mixture, or any means of differentiation better than the study of symptoms under highly artificial conditions, no purpose would be served by an attempt at subdivision beyond the two groups. We must, however, recognize a possibility that strains, indistinguishable by the symptoms they produce, may possess varying virulence for the several cassava types. On this basis we attempted to explain the observation that certain cassavas highly resistant to mosaic in West Africa were susceptible in the Amani district (Storey, 1935); but recent evidence suggests that these same varieties may not possess as high a resistance in West Africa as was at first attributed to them (Lloyd Williams, *in litt.*).

We have encountered no certain evidence that variant strains have arisen spontaneously during the period of our study. Although the fluctuations observed from time to time in symptom expression might be interpreted in this sense, at the end of the observation period each selected strain (or mixture) produced symptoms that were on the average no different from those at the start.

Transmission

General

Bouriquet (1932), Deighton (1935), Hédin (1931) and Lefevre (1935) have reported unsuccessful attempts to transmit a cassava mosaic virus through the soil and the true seed. Without especially studying these points, we have much incidental confirmatory evidence.

All observers agree that the virus is carried in cuttings from diseased plants. Cassava mosaic is somewhat unusual among virus diseases, however, for the frequency with which exceptions to this rule are encountered. In our experience diseased plants have often grown from cuttings taken from an apparently healthy plant (cf. Pascalet, 1932); but we have no reason for attributing this to anything beyond an original failure in diagnosis, due to a too recent infection or to a suppression of symptoms. Apparently healthy plants have grown from cuttings from a diseased plant. In part, this may be attributed to the particular path followed by the virus in moving through the plant (see later). Occasionally, a cutting has produced a healthy plant, while portions of the same stem from above and below this cutting have produced diseased ones. It appears that the virus may not always be fully systemic.

The virus is transmitted across a graft (Deighton, 1932; Pascalet, 1932; Zimmermann, 1906). We have used grafting for several years as a routine technique with consistent success.

The majority of workers have failed to transmit this virus by mechanical inoculation, although successes have been reported by Hédin (1931), Kufferath & Ghesquière (1932) and Lefevre (1935). All our trials have failed, including those where the inoculation was made into immature tissue, which, as we shall show, is alone inoculable by insect vectors. In these experiments mixed juice, strained but not filtered, pressed from plants showing both severe and mild forms of mosaic, was inoculated into 10 Mbarika plants by each of the following methods:

(1) By needle scratch and puncture into the young leaflets.

(2) By rubbing the young leaflets.

(3) By hypodermic injection into the petiole of a young leaf.

(4) By hypodermic injection into the stem near the youngest leaf.

All forty inoculated plants, as well as forty controls, were still healthy two months after the inoculation.

Insect vectors

(1) *General.* Transmission of a cassava mosaic virus by a species of white fly (*Aleurodidae*) was first reported by Ghesquière (1932) in the Belgian Congo; this was confirmed at Amani (Storey, 1934) and in Nigeria by Golding (1936). Other insects are rarely found on cassava in the Amani district. We collected small numbers of three Jassids, *Erythroneura cassavae* China (1930), *Penthimia bella* Stal and *Penthimia vinula* Stal, and bred the first two successfully on cassava. In a limited series of trials, too few to be conclusive, none of these species transmitted a mosaic virus.

Our early trials of the white fly suggested peculiar features in the transmission process by this vector, in that all early experiments (not here recorded) failed if single-leaf cages were used, and success resulted only from general infestation of the plants. Subsequent studies, as detailed below, have revealed the conditions that must be satisfied for successful transmission and enabled us to evolve a reliable and convenient experimental technique.

(2) *Experiments on the conditions for infection.* All experiments were performed in gauze-protected greenhouses. Plants were of the variety Mbarika, and, in all but the earliest experiments, were the vegetative progeny of a single cutting. Control plants were raised either from alternate cuttings from the same stems, or from a random sample of the cuttings. Unless otherwise stated the insects used were wild adult white flies, collected on the same or the previous day on diseased cassava. The experimental plants were recorded as "healthy" or "diseased", without regard to whether the symptoms were severe, mild or intermediate.

The first trial by general infestation was performed by releasing several thousand white flies into a greenhouse compartment containing twenty-eight healthy Mbarika plants. Mosaic symptoms appeared on some plants within a month and after 5 months twenty-seven were diseased. Twenty-eight control plants in an adjacent compartment, not infested with white flies, all remained healthy.

When the plants were caged individually, two practical difficulties were encountered: (*a*) cassava is intolerant of the conditions in a small cage, rapidly becoming oedematous (Wolfe & Lloyd, 1912) and so abnormal that diagnosis of mosaic is difficult, and (*b*) we have found no treatment, not injurious to the plants, that kills the eggs and larval stages of the white fly. The difficulties were overcome by caging young plants (in Dietz lamp glasses) for 1 week only, whereupon the glasses were removed and surviving adult insects captured with a bush dipped

in soap solution. The plants then continued their development in the open greenhouse. Meanwhile larvae of the white fly hatched from eggs laid on the leaves, but since the larvae came to rest nearby, they could be disregarded until the pupae were nearly mature. Therefore, after a further fortnight, all the leaves bearing pupae were cut off, so that a general infestation of the greenhouse by adults was prevented. The plants, having meanwhile produced a crop of new leaves, were not considerably checked in growth by the loss of a few old ones.

Two series of experiments by this method, in which each plant received 50–100 white flies, gave six infections out of eight, and four out of five respectively. Control plants similarly caged and defoliated remained healthy.

The same procedure was now applied to the shoot-tips of older plants, the lamp-glasses being closed below by a large cork, slotted to allow the entry of the stem. One hundred white flies in each glass caused two infections in three plants. In a comparative series on similar lines, shoot-tips and single mature leaves were exposed in the cages. Five out of six plants were infected through the shoot-tip, and none out of six through a mature leaf.

In an experiment to decide which tissue of the shoot-tip could be inoculated successfully by the insects, the lamp-glasses were replaced by glass tubes, 10 cm. long by 1·5 cm. bore: otherwise the procedure was unaltered. When 100 insects were caged by this method with a young leaf less than one-quarter grown, all of five plants became diseased. When the whole shoot-tip was similarly caged, four out of five plants became diseased.

The small glass tube, placed over a young leaf, was used in all later experiments. About 100 insects were introduced into each tube.

The period of exposure of the leaf to the insects was now cut down from the 7 days previously given to 1, 2 and 4 days. The infections obtained were: 1 day, 4 out of 4; 2 days, 3 out of 4; 4 days, 4 out of 4.

In the earlier experiments the exposed leaves had been cut off after two to three weeks from the start. In order to decide whether this delay is necessary for infection, young leaves, after exposure for 2 days to the insects, were cut off after 2, 4 and 8 days from the start. No plant became diseased. The experiment was repeated at intervals of 2, 4, 8 and 16 days from the start; all of the last series and two out of three plants cut at 8 days became diseased, the first two series remaining healthy. We may thus place the threshold period for passage of the virus from the leaf into the stem at near to 8 days.

In order to determine the age at which the leaf ceases to be inoculable by the insect, we exposed a graded series of leaves, from the youngest mature one upwards. The results of this experiment appear in Table I. No infections were obtained through any leaves below the third above the youngest mature leaf. In the third column of Table I appear figures for a mean leaf ratio, representing the proportion of the leaf's full linear growth completed at the start of the experiment. The values rest on the assumption that the successive leaves have equal potentialities for growth, which in plants at the stage selected is near to the truth. In series 4, three plants out of four were infected through leaves with a mean ratio of 0·36. The individual values were: successful, 0·37, 0·37, 0·28; unsuccessful, 0·42. There is, thus, an indication that the critical stage is reached at near to 0·4. In practice however we have adopted 0·25 as a safe figure.

Table I. *Age at which cassava leaves cease to be inoculable by the insect*

Series no.	Type of leaf exposed	Mean leaf ratio and standard error*	No. of plants exposed	No. of plants infected
1	Youngest mature leaf	1	4	0
2	First above youngest mature leaf	0·87 ±0·023	4	0
3	Second above youngest mature leaf	0·70 ±0·037	4	0
4	Third above youngest mature leaf	0·36 ±0·029	4	3
5	Fourth above youngest mature leaf	0·20 ±0·029	4	4

* Ratio calculated for each plant as

$$\frac{\text{Length of middle leaflet of exposed leaf}}{\text{Length of middle leaflet of youngest mature leaf}}.$$

(3) *A successful technique.* In the course of the preceding studies we evolved a technique that is convenient, economical and reliable. We give below exact details of the procedure.

(i) Choose a leaf not more than one-quarter grown (Text-fig. 1*a*), shorten its leaflets if necessary and cut off the shoot-tip above this leaf (Text-fig. 1*b*).

(ii) Wrap the base of the petiole with wool and place over it the glass-tube (about 10 by 1·5 cm. bore) (Text-fig. 1*c*). Secure the tube by a rubber band to an upright stick.

(iii) Blow in the insects from a pipette, connected by a rubber tube to a short length of glass-tubing passing through the upper wool plug (Text-fig. 1*c*). This glass-tubing may now be withdrawn, the hole through

which it passed closing naturally by the resilience of the wool. Shade the tube from direct sunlight.

(iv) After two days remove the plant from the greenhouse, withdraw the tube gently and capture all adult insects with a bush dipped in soap solution. The exposed leaf must not be injured during this treatment. Return the plant to the greenhouse.

(v) After a further 14 days cut off the leaf at the base of the petiole.

Text-fig. 1. Technique for experiments in transmission of cassava-mosaic virus by white flies. *a*. Shoot tip at a suitable stage for experiment. The next to the youngest leaf is about one-quarter grown. ×2. *b*. The same shoot tip, with the youngest leaf and growing point removed and one older leaf removed. Leaflets of the selected leaf shortened. ×2. *c*. Tube placed over the selected leaf, with wool plugs and small piece of glass tubing for introducing the insects. Support for the tube is not shown. ×1.

Under our greenhouse conditions, symptoms of mosaic have begun to appear in the new terminal growth after 12–20 days from the start. The method is effective with other varieties besides Mbarika. Ten plants each of Malindi and Mpezaze, varieties reputed to be more than normally resistant in the field, were exposed to 100 white flies each by this method. Nine plants of each variety were infected.

(4) *Transmission of different virus strains.* The insects used in the preceding experiments, having been collected on diseased plants in the field, were likely to be carrying both severe and mild strains of the virus. Although, once they were certainly diseased the plants were usually not further studied, the majority showed severe symptoms, and there could be no doubt of the insects' ability to transmit a severe strain of the virus. A few plants, however, developed mild symptoms suggesting that the insects had here transmitted only a mild strain. Their ability to do so was confirmed in the following experiments.

A mixed non-infective culture of white flies was established by repeated transfers to healthy plants of a *Phaseolus* sp. A test of a sample of these insects gave no infections in six cassava plants[1]. Insects from this culture were transferred to an Mbarika plant carrying the D 2 mild virus strain, and allowed to develop for 6 months. A test of this second culture gave six infections in six Mbarika plants, all showing the typical symptoms of the D 2 strain.

(5) *Identity of the vectors.* Ghesquière (1932) referred the vector in the Belgian Congo to his *Bemisia mosaicivecta* sp.n. (a misprint, as he informs us by letter, for "*mosaicivectura*"). Later (1934), Mayné and he listed this insect under the designation *B. gossypiperda* Misra and Lamba var. *mosaicivectura*. Specimens from a field collection at Amani submitted to M. Ghesquière were determined as identical with his.

Three inbred cultures of the insects were raised on *Phaseolus* spp. from single wild females. Their ability to transmit was tested in three greenhouse compartments, containing surface-sterilized cuttings of Mbarika planted in steam-sterilized soil. Each insect culture was established on diseased plants in each compartment, and left to migrate naturally to the healthy plants. The results were: Culture A caused infection of seven out of ten plants; culture B, two out of ten; and culture C, none out of ten. Control plants in a compartment adjacent to A remained healthy and free from white flies.

Specimens of culture A were submitted to Mr Karam Singh Lamba (one of the authors of *Bemisia gossypiperda* (Misra & Lamba, 1929)), and, through the Director of the Imperial Institute of Entomology, to Mr G. H. Corbett. Mr Karam Singh reported that our material appeared

[1] Very few of the insects survived the two days of this test. This has been our usual experience when this white fly is transferred to a new species of food-plant. The control test is therefore not conclusive, but does not detract from the evidence that the white fly can transmit a mild virus strain.

to him to be *B. gossypiperda*. Mr Corbett identified it as *Bemisia* sp. near *nigeriensis* Corb.

Golding (1936) refers the insects of his trials to *B. nigeriensis* Corb.

In view of the difficulties that this group of insects presents to the systematists, it remains in doubt whether one or several species of *Bemisia* transmit the virus of cassava mosaic; and, indeed, whether the same or different species transmit certain other African viruses affecting tobacco (Storey, 1931) and cotton (Kirkpatrick, 1930).

Dissemination of the virus through the plant

We have studied the spread of the virus through a recently infected plant by growing, as cuttings, sections of its stem. Twenty-eight plants, selected in an experimental plot as having developed during the preceding month the first symptoms of mosaic, were thus found to have virus right to the base of the diseased shoots, even though some were as long as 8½ ft. Fifteen of the plants bore also apparently healthy shoots arising from the same original cuttings; six of these shoots were free from virus, seven contained virus throughout their length, and two gave diseased plants from their lower cuttings and healthy ones from the upper cuttings. Although the virus travels down to the stem rapidly, its movement across the original cutting and into other shoots may be relatively slow.

Similarly, the movement into side branches borne on the infected shoot appears to be slow. One or two apparently healthy branches borne by eleven otherwise diseased shoots were tested, and all were found to be virus-free except those from four shoots. One of these gave diseased plants from the lower cuttings and healthy from the upper, and three gave all diseased plants. Since all branches of the original plants were exposed to an equal chance of natural infection, some of the last may have been infected independently before cutting, although too recently to have developed symptoms. There is, evidently, a marked tendency for the virus to move down a shoot, passing on its way the origin of a branch and yet failing to move up into it. On one plant the virus-free branch arose within 2½ ft. of the top of a shoot 8½ ft. long, which contained virus right to its base.

Interaction of virus strains

Recent work (Kunkel, 1934; Salaman, 1933; Thung, 1931) has shown that a mild strain of a virus can sometimes confer immunity from a severe strain. The mild strains of the cassava mosaic virus here studied,

however, gave no evidence of conferring immunity when a severe strain was later introduced by grafting. A large number of experiments were performed, in which stocks of the variety Mbarika, previously infected by grafting with the mild strains D 2, D 7, D 8 and D 11, received scions carrying a severe strain. In every experiment symptoms of the severe strain appeared in the new growth from the stock. We may note, however, that Köhler (1935) has reported the breakdown of an otherwise effective immunity, when the manner of infection was through a graft.

When the severe strain was introduced by the insect vector, some indication of an interaction was obtained. In our first experiment, five Mbarika plants carrying the mild D 2 strain were exposed by the usual technique to infection by wild white flies. Five healthy Mbarika plants were similarly treated. These control plants all became diseased, and all but one showed typical severe symptoms. Of the five D 2 plants, two never showed more than the faint symptoms of the D 2 virus; one developed the severe disease and was indistinguishable from the controls; while two plants produced leaves with only small areas of severe chlorosis scattered over their surface, on which the mild symptoms of D 2 were evident (similar to Pl. XXXII, fig. 3). The one exceptional control plant was also of this type. All these plants were retained under observation for 9 months, during which time the general type of symptoms in each underwent no marked change.

When this experiment was repeated, none of 7 plants originally carrying the D 2 virus escaped infection by a severe strain. Two developed a severe disease and 5 severe flecks on a mild background (Pl. XXXII, fig. 3). The originally healthy control plants, however, showed closely similar symptoms, only one having the typical severe disease.

An evident defect in our experimental procedure was the use of wild insects, liable to be carrying both severe and mild virus strains. It is possible that the anomalous results obtained, particularly in the last experiment, may have been due to a simultaneous infection by two strains. The experiments have, however, demonstrated that no reliance can be placed on the mild strains at present available as a practical means of protection from severe strains. Field trials with cassava carrying the D 2, D 7, D 8 and D 11 strains confirmed this conclusion. Although these trials permitted no exact comparison of resistance between healthy and mildly diseased clones, they showed that all the mildly diseased ones could contract naturally the severe disease.

Discussion

We have shown that mosaic disease of cassava is not due to a single uniform causative agent. It is hardly important whether, at this stage, we regard the agents as different viruses or strains of one virus. We have chosen the second course as conforming best with the modern outlook on the virus problem. The proof that one species of insect transmits viruses causing both the severe and the mild forms of the disease favours this choice. The fact that a mild strain had failed to confer immunity from a severe one does not point necessarily to a contrary choice. We have shown that there is a marked tendency for the symptoms of cassava mosaic to be confined to limited areas of the leaf surface. This may denote a similar localization of the virus. In the modern view (Salaman, 1936), protection by one strain of virus from another depends on "occupation" of the tissues; plant cells that are occupied by one strain are unavailable to another. Thus, we may suggest that in cassava a mild strain of virus may occupy only defined areas of the leaf, leaving other areas open to invasion by a severe strain. We have observed that, in a mixed infection, the symptoms of the severe disease tend to be more circumscribed than in a single infection by a severe strain alone.

Although we have reason for dividing the strains of the cassava mosaic virus into two groups and may suspect differences between members in each group, we have only touched the fringe of this problem. At present we have only a hint of the existence of strains indistinguishable by symptom examination but differing in their ability to infect the several cassava types. And yet this is the important practical problem, for it may well decide whether the extensive work of breeding for resistance now in progress will have any permanent and widespread success. But this same breeding programme may provide us with the differential hosts that are the essential tools of a sound study of virus strains.

Our study of insect transmission has revealed a situation that, for its dependence on certain rigid conditions, is probably unique. We can offer no explanation why an insect, that can successfully maintain itself on mature leaves, can inoculate successfully only immature ones. We can perhaps perceive a reason why, after inoculation, these leaves need several days to develop before the virus will pass out of them into the stem. There is a growing tendency to relate the movement of certain viruses within the plant to the translocation of carbohydrates (cf. Bennett, 1937). We may suppose that the young leaf, at the inoculable stage, is

receiving carbohydrates from other parts of the plant, and that only when its photosynthetic mechanism becomes active and the flow of carbohydrates is reversed, can the virus travel from the leaf into the stem. On similar grounds we may, perhaps, explain the rapid movement of the virus down the stem and its slow upward movement into branches.

Our insect-transmission experiments, largely successful once technical difficulties were overcome, have all been by mass infection with large groups of insects. We have no reason for attributing any particular merit to the group except that of increasing the probability of infection. We have, however, at present no conclusive evidence that a single insect is capable of inoculating a plant successfully.

Summary

The symptoms of mosaic disease in cassava, although generally typical of the mosaic group, show wide variations, due in part to the varietal reaction of the plant, to its stage of development and to the environment. The most important cause of variations however is differences in the strains of the virus, of which we have recognized, through the study of symptoms, two groups of severe and mild strains.

The viruses are transmitted across a graft, but we have failed to obtain mechanical transmission by needle or hypodermic injection.

A *Bemisia* sp. can transmit both groups of strains. It can inoculate the plant only through immature leaves, less than about one-quarter of their full length. The virus so inoculated does not pass out of the leaf until about 8 days have elapsed. On the basis of this knowledge a convenient and reliable single-leaf cage technique has been developed.

After the virus has entered the stem it passes rapidly to the base of this stem, but only slowly into side branches from it or into other stems arising from the same original cutting.

Infection of a plant with a mild strain of virus failed entirely to confer immunity from infection by severe strains introduced by grafting. If the severe strains were inoculated by insects there was an indication of some conferred resistance but insufficient to make the procedure practically useful in control.

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EXPLANATION OF PLATES XXXI AND XXXII

PLATE XXXI

Fig. 1. A severe strain of the mosaic virus (no. G 1). Typical manifestation in var. Mbarika in the greenhouse. $\times \frac{2}{3}$.

Fig. 2. A mild strain (no. D2). Typical manifestation in var. Mbarika in the greenhouse. $\times \frac{1}{12}$.

[N.B. Figs. 1 and 2 are from strictly comparable leaves. That in fig. 2 is about the normal size for a healthy leaf of this variety; the reduction in size of the leaves in fig. 1 is a typical effect of the virus strain.]

Fig. 3. The shoot-tip of a diseased plant, bearing in succession (*a*) severely diseased leaves, (*b*) one leaf with severe chlorosis confined to the base of each leaflet, (*c*) a leaf entirely healthy except for a few chlorotic flecks, (*d*) a further group of severely diseased leaves. $\times \frac{1}{3}$.

PLATE XXXII

Fig. 1. A mild strain (no. D11). An example of the most severe manifestation given by this strain in var. Mbarika in the greenhouse. Under similar conditions the strain D2 rarely gives symptoms as severe as this. $\times \frac{1}{3}$.

Fig. 2. A mild strain (no. D2). The manifestation shown by var. Mbarika grown in the cool season in a valley at Amani subject to cold-air drainage. Cuttings from this plant showed the typical D2 symptoms (as fig. 2 Pl. XXXI) when grown in the greenhouse. Plants with the D11 strain growing alongside in the valley were at this time almost symptomless. $\times \frac{1}{3}$.

Fig. 3. A mild strain (no. D2) combined with a severe strain. A leaf from a plant, originally infected with the D2 strain, later exposed to infection by wild White Flies. The severe symptoms are confined to small areas of severe chlorosis, between which the mild symptoms of D2 can be faintly seen. $\times \frac{1}{3}$.

Fig. 4. A severe strain showing an extreme localization of effect to one leaflet and a part of one other. The remaining leaflets appear to be entirely healthy. $\times \frac{1}{3}$.

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Fig. 1.

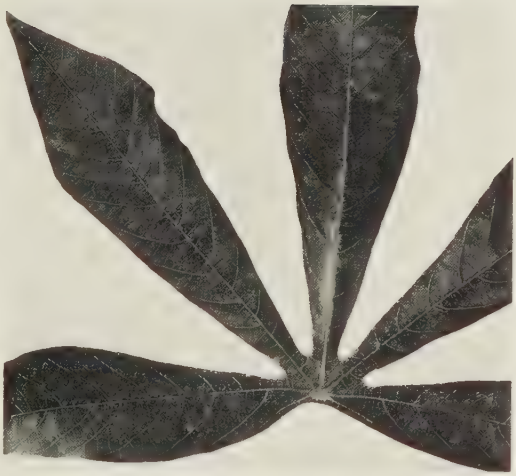

Fig. 2.

Fig. 3.

STOREY AND NICHOLS.—MOSAIC DISEASES OF CASSAVA (pp. 790–806)

Fig. 2.

Fig. 4.

Fig. 1.

Fig. 3.

STOREY AND NICHOLS.—MOSAIC DISEASES OF CASSAVA (pp. 790–806)

STUDIES ON AMERICAN FOUL BROOD OF BEES

III. THE RESISTANCE OF INDIVIDUAL LARVAE TO INOCULATION WITH THE ENDOSPORES OF *BACILLUS LARVAE*

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In previous experiments it was found that colonies of bees contract American foul brood rapidly when inoculated with the spores of *Bacillus larvae*, while the vegetative cells fail to produce the disease (Tarr, 1937*a*). In these experiments the larvae were not inoculated individually, the spores or vegetative cells of *B. larvae* being either fed to the bees in syrup or sprayed over the combs of developing larvae. Such methods of inoculation are unsatisfactory for the reasons that, although direct inoculation of larvae may take place by the spraying method, both techniques present the possibility for indirect inoculation of the brood, as the causal organism is carried by adult bees; and because neither of them allow for an estimation of the minimum lethal dose of spores for a single larva. Experiments designed to ascertain the minimum lethal dose of spores by oral inoculation of individual larvae yielded the results recorded below.

Experimental

Suspensions of washed spores of *B. larvae* were prepared from ropy larvae dead of American foul brood as previously described (Tarr, 1938*b*). Two to five comb nuclei of hybrid bees prepared as in former experiments were used (Tarr, 1936). The method of inoculation of individual eggs or larvae was essentially an oral one. Combs containing eggs or brood of the age desired were removed from the colonies. From 0·001 to 0·005 ml. of spore suspension was introduced into the larval cell by means of an Agla micrometer syringe, being either placed at the base of the cell near the eggs, in the brood food surrounding the younger larvae, or near the mouth parts of the older coiled larvae. In a few instances spores were fed to recently sealed larvae by introducing the needle of the hypodermic syringe through the cell cap. The rows of cells containing the inoculated larvae were marked by driving large pins through the midrib of the combs

containing them, and the combs were returned to the bee colony immediately. It was found that, with careful inoculation, adult bees rarely remove young larvae which are fed approximately 0·001 ml. of suspension, or older larvae which are fed up to 0·005 ml. With larger volumes of inocula there is great danger that the bees will remove numbers of the inoculated larvae. In certain of the experiments the spores were sprayed over the developing brood by the technique already described (1937*a*). Periodical examinations of the inoculated larvae were made, and, in many instances, a certain number of pupae were selected at random from the inoculated areas and examined microscopically. The method of recording inoculations and results was similar to that employed in previous work (Tarr, 1936, 1937*a*). A large number of larvae in many different colonies were inoculated but only a limited number of typical experiments are recorded here. No attempt has been made to calculate the standard error of the spore number in the case of each inoculum for the reason that, as has already been shown (Tarr, 1938*b*), it is unlikely to exceed $\pm 5\%$ with spore suspensions containing over 1000 spores per ml. with good counting and dilution methods.

Exp. 1. Date of inoculation 25 June.

Each of twenty-five eggs and young larvae about 1 day old, in four separate groups in adjacent cells, were fed 0·005 ml. of spore suspensions of *B. larvae* so that the individual larvae in the various groups received approximately 44×10^2, 44×10^3, 44×10^4 and 44×10^5 spores respectively.

Results. 6 *days*: A number of inoculated larvae had been removed by the bees (probably about 25%), the remainder had been sealed over and no infected larvae were seen. 8 *days*: No diseased larvae were seen. Three sealed pupae from each group were selected at random and were examined microscopically: no spores or vegetative cells of *B. larvae* were detected in them. Subsequent examinations 18, 22, 27 and 32 days after inoculation revealed no infected larvae, the colony remaining free from American foul brood.

Exp. 2. Date of inoculation 28 June.

Individual larvae about 1 day old and eggs in three groups of sixty were fed 0·005 ml. portions of spores suspensions so that each received approximately 10×10^4, 10×10^5 and 10×10^6 spores respectively.

Results. 3 *days*: Very few larvae had been removed by the bees, and most were ready for sealing; no diseased larvae were seen. Further examinations of the brood 9, 21, 37 and 65 days after inoculation revealed no infected larvae.

Exp. 3. Date of inoculation 28 June.

Individual larvae 4–5 days old in three groups of forty were inoculated with doses of spores of the same magnitude as those given in the previous experiment.

Results. 1 *day*: The inoculated larvae were being sealed by the bees and practically none had been removed. Two pupae selected at random from each group were examined

microscopically. A few spores were seen in those which had received the larger inocula, but there was no sign of germination of these, and no vegetative cells of *B. larvae* were detected. 3 *days*: Few if any of the inoculated larvae had been removed by the bees. Three pupae from each inoculated group were selected at random and examined microscopically. All appeared to be free of spores of *B. larvae*; no bacteria being seen in any of the nine preparations examined. No larvae affected with American foul brood were seen on examinations 9 and 18 days after inoculation. The colony swarmed 19 days after inoculation and was permitted to requeen itself. The brood produced by the new queen was examined 65 days after inoculation and proved to be healthy.

Exp. 4. Date of inoculation 8 July.

Eggs and young larvae about 1 day old in three adjacent groups of forty were fed 0·001 ml. of three different dilutions of a spore suspension of *B. larvae*, so that individual larvae received approximately $9{\cdot}4 \times 10^4$, $9{\cdot}4 \times 10^5$ and $9{\cdot}4 \times 10^6$ spores respectively.

Results. 3 *days*: Very few inoculated larvae had been removed by the bees and many were practically ready for sealing. Subsequent examinations 5, 11, 20, 26 and 53 days after inoculation revealed no larvae affected with American foul brood.

Exp. 5. Date of inoculation 20 July.

One-hundred, 4–5 day old unsealed larvae, and a few recently sealed larvae, were fed 0·005 ml. containing approximately 47×10^6 spores each.

Results. The larvae in the inoculated area had all been capped by the bees and few if any had been removed. Ten sealed pupae were selected at random from the inoculated portion of the comb. On microscopical examination all appeared to be free of spores or vegetative cells of *B. larvae*. 8 *days*: No infected larvae seen. 12 *days*: A few of the bees in the inoculated area emerging and apparently healthy; no diseased larvae seen. No sign of American foul brood was observed on inspection of the brood 20, 41 and 50 days after inoculation.

The results of the above experiments indicate that individual larvae possess a very high degree of resistance to simple oral inoculation with the spores of *B. larvae*. It was thought that some essential substance might have been removed on washing the spores and therefore direct inoculation with whole crushed infected larvae and also with unwashed spore suspensions were tried.

Exp. 6. Date of inoculation 19 July.

Fifty individual larvae about 4–5 days old were each fed approximately $7{\cdot}8 \times 10^5$ spores of *B. larvae* in 0·005 ml. of an unwashed suspension.

Results. 4 *days*: Few if any of the inoculated larvae had been removed by the bees and no diseased larvae were seen. Six sealed pupae from the inoculated area of the comb were selected at random, and microscopical examinations of these showed no bacteria. No sign of American foul brood was found when the colony was examined 8, 13, 21, 38 and 51 days after inoculation.

Exp. 7. Date of inoculation 28 July.

Four larvae very recently affected with American foul brood which, on microscopical examination, were found to contain practically nothing except non-sporulating vegetative cells of *B. larvae* in apparently pure culture, were ground up in 5 ml. of

distilled water. Approximately 0·005 ml. of the resulting suspension were fed to each of fifty, 4–5 day old larvae.

Results. 3 *days*: A few of the inoculated larvae had been removed by the bees and the remaining sealed brood showed no infected larvae. Subsequent examinations 6, 12, 29, 35 and 41 days after inoculation revealed no infected larvae.

In view of the failure which had accompanied all attempts to inoculate individual larvae by direct oral inoculation with relatively massive doses of *B. larvae* spores, experiments were made in order to verify previous work in which it was shown that spraying dilute spore suspensions over developing brood causes rapid spread of American foul brood (Tarr, 1937*a*). The results obtained in a typical experiment are given below.

Exp. 8. Date of inoculation 15 July.

One comb containing chiefly eggs and young larvae was removed from a three comb nucleus and the brood was sprayed with 20 ml. of an aqueous suspension containing approximately 94×10^6 spores of *B. larvae*.

Results. 7 *days*: The inoculated comb contained a number of rather dull white, flabby, chewed pupae and two slimy brown pupae. Ten of these were examined microscopically and all contained numerous vegetative cells (some sporulating) or spores of *B. larvae*, and from four this organism was isolated in pure culture. Further examinations 11 and 19 days after inoculation showed that American foul brood was spreading rapidly, and the colony was destroyed.

The results of these experiments strongly suggested that the adult bee was playing some part in transferring the disease to the larvae, and some experiments were made in an attempt to ascertain whether *B. larvae* increased in virulence during passage through the adult bee.

Exp. 9. Date of inoculation 5 August.

A number of bees which had sucked up the ropy decaying remains of larvae dead of American foul brood when streaked over the surface of a brood comb were caged for 24 hr. at 33° C. Their recta were full of spores of *B. larvae*. Four of the bees thus treated were ground up as finely as possible in 5 ml. of *M*/5 phosphate buffer *p*H 7·2. Fifty 4–5 day old larvae were each fed approximately 0·005 ml. of this suspension (the coarse debris was allowed to settle for a few minutes before the hypodermic syringe with which the larvae were inoculated was filled).

Results. 4 *days*: No diseased larvae were noticed in the inoculated area of brood comb. Nine sealed pupae from this area were examined microscopically. All but two of these were apparently free from bacteria. The remaining two showed a few rod-shaped bacteria but it is not known whether these were *B. larvae* organisms. All subsequent examinations carried out at intervals of 11, 20, 27 and 34 days after inoculation showed that the brood of the colony remained absolutely free from American foul brood.

Exp. 10. Date of inoculation 26 August.

Twenty-five young bees were taken from a healthy colony and were fed as much of a mixture containing equal portions of honey and ropy material from larvae dead of American foul brood as they would take in 24 hr. at 33° C. Eight of the bees thus treated were ground up finely in 5 ml. of sterile distilled water. The coarse debris in the resulting suspension was removed by filtering it through sterile cheese cloth. Twenty-

five 4–5 day old larvae and twenty-five 1–2 day old larvae were fed 0·005 and 0·001 ml. portions of this suspension respectively.

Results. 4 *days*: Nearly all the inoculated larvae had been sealed by the bees, very few having been removed. After 6 and 13 days the brood was again examined but no infected larvae were seen.

Similar experiments made with either ground up recta or whole digestive tracts of adult bees taken from colonies badly affected with American foul brood yielded equally negative results.

Discussion

The direct inoculation of individual larvae has never proved a satisfactory method of introducing American foul brood to a colony of bees. On the other hand, European foul brood is initiated readily by this method (Tarr, 1938*a*). White (1920) stated that, "Direct inoculation (of larvae) with a capillary pipette is less satisfactory in experiments on American foul brood than in those on Sacbrood", but did not describe the experiments, the results of which led him to make this statement, in detail. Toumanoff (1929) was unable to infect individual larvae by feeding them directly with vegetative cells obtained from pure cultures of *B. larvae*. In his experiments he records that over one half the inoculated larvae were removed by the bees while the remainder matured normally. Sturtevant (1932) attempted to inoculate individual larvae, at ages ranging from about 4 days up to the time just subsequent to the sealing of their cells, by feeding them 0·01 ml. portions of sugar syrup containing *B. larvae* spores. In his experiments he returned the combs containing the inoculated larvae to the bee colonies in such a way as to keep them from contact with the adult bees for some time, thus introducing a short period of "starvation" of the larvae. With the method of inoculation used, he found that the bees invariably removed larvae which were fed approximately 25×10^6 or 50×10^6 spores, while those receiving smaller numbers were removed less frequently, depending on the length of time the larvae were kept away from the adult bees and the size of inoculum of spores. In only one of a series of nuclei used was American foul brood initiated by this method of inoculation and, in this case, the individual larvae had been fed approximately 10×10^6 spores. It seems highly likely that in this case the susceptibility of the individual larvae to infection with *B. larvae* spores was enhanced by the starvation period, for the writer showed that it is fairly easy to cause an infection in healthy larvae by feeding them large doses of certain of the secondary invading bacteria occurring in European foul brood if the larvae fed are subsequently starved at hive temperature (Tarr, 1936). When larvae thus

inoculated were returned to a healthy bee colony no disease resulted. From his experiments Sturtevant concluded that it is by no means easy to infect individual larvae with American foul brood, but did not suggest that the adult bee might play a very important part in disseminating the disease within the bee colony.

Jaeckel (1930) investigated the histological changes which occur in larvae affected with American foul brood. Only a few of the larvae which he studied were naturally infected, the majority being inoculated by placing 0·1–0·15 ml. of a suspension made from ropy larvae dead of American foul brood mixed with one or two parts of water in their cells. This method of inoculation would seem to be very crude since the average worker cell holds approximately 0·25 ml. of water, and the volume of inoculum he employed must have covered and probably chilled the larvae. Indeed, he remarks that in some experiments all the inoculated larvae were removed by the bees and, at the best, only about 20% were not removed. It is surprising that such a drastic method of inoculation ever succeeded, for the writer, in somewhat extensive experiments, has found that there is a strong tendency for the bees to remove larvae, especially the younger ones, when volumes of inocula much in excess of 0·01 ml. are employed. Jaeckel stated that he kept the combs containing inoculated larvae for a few hours at 34–37° C., until the larvae had consumed the infective material, before introducing them to bee colonies. It is practically certain that unsealed larvae would never consume a fraction of the large dose of suspension which was fed to them. From the results of his histological work Haeckel was led to state that American foul brood is inclined to be a type of septicaemia, the causal organism penetrating the epithelial cells of the stomach of the larvae and being carried by the blood to various organs. If this be so, then this disease must differ radically from European foul brood which is apparently a purely intestinal infection, the causal organism being localized in the larval gut (Tarr, 1938*a*). It is of interest that Jaeckel realized that such large inocula as he employed were extremely unlikely to occur under natural conditions, and he suggested that *B. larvae* might gain access to the larva by some route other than the oral one. Thus, he mentioned that the spiracles of the larva might offer a convenient portal of entry to the causal organism.

When the results of the work discussed above are considered in conjunction with those obtained in the present experiments it is evident that some factor exists by which the pathogenicity of *B. larvae* for its host is determined. Since indirect inoculation, by allowing the adult bees

of the colony access to dilute spore suspensions of *B. larvae*, is uniformly successful in producing American foul brood, and direct inoculation of individual larvae at all stages of growth with concentrated spore suspensions fails to cause the disease, it can only be inferred that the adult bee plays an important part in inoculating the brood of the bee colony. This role might be a purely physical one, the bee introducing spores of *B. larvae* into the larva in some manner which is not attained by simple oral inoculation; or a more complex one, the pathogenicity of the spores being changed by the adult bee. Careful histological work, such as has been carried out on larvae affected with European foul brood (Tarr, 1938*a*), might yield much valuable information regarding the manner in which *B. larvae* first invades the tissues of its host. The possibility that *B. larvae* undergoes some change, during its carriage by the adult bee, which causes it to become more virulent must be considered. So far experiments designed to test this point, which have been recorded in this paper, have yielded negative results. However, further experiments should be carried out in order to determine the fate of *B. larvae* spores in adult bees of various ages, particularly in those bees which have assumed the nursing duties of the colony. The significant part played by insect vectors in the transmission of virus diseases of plants has long been recognized. Thus streak disease of maize, which cannot be transmitted from plant to plant by simple mechanical means, is readily carried by certain species of leaf hoppers inoculated with the causal virus (Storey, 1928, 1933). It will be interesting if the adult bee proves to be an "insect vector" to its own brood.

Summary

American foul brood is not produced by direct inoculation of eggs, or larvae from the time of hatching to that just subsequent to sealing, by placing from 0·001 to 0·005 ml. of aqueous suspensions of washed spores of *Bacillus larvae*, obtained from natural sources, in their cells.

With this method of oral inoculation, larvae are rarely removed by the bees and mature normally when each receive from a few thousand to over 45×10^6 spores of *B. larvae*.

Feeding individual larvae with unwashed spores of *B. larvae*, or with portions of crushed larvae recently affected with American foul brood, does not cause the disease.

Methods of inoculation, in which the adult bees are allowed access to relatively dilute suspensions of *B. larvae* spores, form a satisfactory means of initiating American foul brood in colonies of bees.

It is inferred that adult bees play an important part in carrying American foul brood in the colony, and the possible role of the adult bee as "insect vector" of American foul brood is considered.

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STUDIES ON EUROPEAN FOUL BROOD OF BEES

IV. ON THE ATTEMPTED CULTIVATION OF *BACILLUS PLUTON*, THE SUSCEPTIBILITY OF INDIVIDUAL LARVAE TO INOCULATION WITH THIS ORGANISM AND ITS LOCALIZATION WITHIN ITS HOST

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(With Plates XXXIII–XXXV)

Previous experiments verified the fact that European foul brood is a single disease caused by *Bacillus pluton* White, an organism which refused to multiply on any of a variety of nutrient substrates tested (Tarr, 1936). It was also found that the gross external symptoms of the disease could be greatly modified by introducing large numbers of certain of the bacteria which commonly occur as "secondary invaders" in this disease into recently affected colonies. It seemed important to ascertain whether *B. pluton* would grow upon the tissues of the developing chicken embryo, the minimum lethal dose of this organism for individual larvae, and by what method it invades the tissues of its host. These points have been investigated experimentally and the results obtained are recorded below.

Experimental

(i) *Attempts to cultivate* B. pluton *on the tissues of the developing chicken embryo*

The chorioallantoin membrane and embryo brei media were prepared as in experiments concerned with the growth of *B. larvae* (Tarr, 1938*a*). Suspensions of *B. pluton* were made from the stomachs of larvae recently affected with European foul brood by the technique previously described (Tarr, 1937), Tyrode or Ringer solution being used as suspending medium in most cases instead of phosphate buffer. *B. pluton* tends to form clumps, and although the larger clumps can frequently be removed by passing the suspension through a very thin layer of sterile cotton wool, it is practically impossible to obtain uniform suspensions of this organism as with *B. larvae* spores (Tarr, 1938*a*). The addition of a trace of sodium hydroxide solution together with some methylene blue facilitates the direct counts of

the organisms in such suspensions by tending to break up the clumps and to stain the cells, but such treatment cannot be used for suspensions employed for inoculation. Consequently, it has only been possible to give approximate figures for the numbers of organisms used for a given inoculation, and the figures recorded in the following tables must only be considered as such. All cultures were incubated at approximately 35° C.

Exp. 1. Four tubes containing 5 ml. of embryo brei medium were inoculated with *B. pluton* organisms, two tubes receiving approximately 40×10^6 organisms in 1 ml. of suspension, and the other two 40×10^5 in 0·1 ml. The inoculated media were incubated, microscopical examinations being made at intervals. *B. pluton* organisms could readily be demonstrated in the freshly inoculated medium, but these rapidly disappeared, perhaps owing to the lytic action of the medium or to an actual autolysis. After 3 weeks examination revealed no bacterial cells, and transfer of portions of the inoculated brei to beef digest brood filtrate agar resulted in no bacterial growth.

Exp. 2. The stomachs of four apparently healthy larvae about 3 days old which contained *B. pluton* in apparently pure culture were obtained, each gut being suspended in approximately 0·1 ml. of Tyrode solution. Each suspension was introduced separately into 5 ml. of embryo brei medium, and the resulting cultures were incubated as usual. After 1 day *B. pluton* organisms could be seen in large numbers in three of the inoculated tubes, but there was no sign of increase. The remaining tube of medium was contaminated by an organism which, on cultivation on other media, proved to be the so-called *Streptococcus apis*. Further examinations of the remaining tubes showed that *B. pluton* rapidly disappeared, and all three were apparently sterile bacteriologically after 30 days' incubation.

Exp. 3. The chorioallantoin membranes of three 13-day-old chicken embryos were slightly scratched, and were then inoculated by placing 0·1 ml. of a suspension containing approximately 4×10^6 *B. pluton* organisms on them. One membrane became contaminated by a torula. On the remaining two *B. pluton* refused to develop, the cells slowly disappearing. The chicken embryos continued to live and develop until they were about 18–19 days old, but died prior to the normal hatching time. *B. pluton* organisms could not be demonstrated either in the chorioallantoin membrane, or heart, liver, spleen or brain of the embryos.

Exp. 4. The whole stomachs of three apparently healthy larvae which contained *B. pluton* in apparently pure culture were placed on the chorioallantoin membranes of three 12-day chicken embryos. One embryo was also inoculated by driving a sharp needle through the chorioallantoin membrane. After 3 days' incubation the eggs were examined. One embryo (the one which had been inoculated directly) was dead and the whole contents of the egg were full of sporulating *B. alvei* organisms. The remaining two embryos were still alive and showed no *B. pluton* cells. Subsequently, one embryo died about 2 days prior to the normal time of emergence apparently free from bacteria, while the other matured normally and emerged from the inoculated egg.

Exp. 5. A 9-day-old embryo was inoculated directly through the chorioallantoin membrane with approximately 1×10^6 *B. pluton* organisms in 1 ml. of liquid. Examinations of the fluid beneath the chorioallantoin membrane and of the scrapings from the membrane itself, carried out at intervals during the 8 days following inoculation, showed that the bacterial cells rapidly disappeared.

Exp. 6. The chorioallantoin membrane of a 7-day-old embryo was inoculated with the greater portion of the stomach of a larva recently affected with European foul brood and in which *B. pluton* was present in apparently pure culture. The remaining portion was streaked on to the surface of a tube of beef digest brood filtrate agar, and no bacterial growth resulted after 1 week's incubation. The inoculated embryo was watched and was still alive after 10 days' incubation. Smears made from the chorioallantoin membrane and from the organs of the 17-day-old embryo showed no *B. pluton* organisms or other bacteria.

The results of the above experiments show that *B. pluton* will not grow on the minced tissues of the chicken embryo, on its chorioallantoin membrane or even in the embryo itself.

(ii) *Inoculation of individual larvae with* B. pluton

Suspensions of *B. pluton* in either Tyrode solution or phosphate buffer were prepared from the stomachs of larvae recently affected with European foul brood by the technique already described. These were prepared and suitably diluted with either Tyrode solution or phosphate buffer on the same day as the inoculations were made. Two or three comb nuclei of hybrid bees prepared as in previous experiments (Tarr, 1936) were used for the inoculations.

Exp. 1. A suspension was prepared containing approximately 40×10^6 *B. pluton* organisms per ml. of phosphate buffer. This suspension was tested for purity as follows: 0·5 ml. was inoculated on to the surface of a tube of beef digest brood filtrate agar; no growth resulted after the culture was incubated for 3 weeks. 0·5 ml. was placed on the chorioallantoin membrane of a 10-day chicken embryo. The embryo developed normally until a few days prior to the time of hatching. When examined *B. pluton* could not be detected in any of its organs.

On 2 July a comb containing chiefly eggs and young larvae was removed from a healthy nucleus and three groups of 100 young larvae about 1 day old in adjacent rows of cells were fed 0·001 ml. portions of the above suspension, or of dilutions prepared from it, so that each larva received approximately 40,000, 4000 or 400 *B. pluton* organisms respectively. The infective material was placed in the brood food surrounding the larvae. The comb was returned to the colony and larvae were removed at random from the inoculated area 2 and 3 days after inoculation, and their intestines examined microscopically for *B. pluton*. The results are given in Table I.

Table I. *Results of oral inoculation of individual larvae with* B. pluton

Approximate no. of *B. pluton* organisms fed	No. of larvae showing *B. pluton* cells in their stomachs			
	2 days after inoculation		3 days after inoculation	
	No. of larvae examined	No. showing *B. pluton*	No. of larvae examined	No. showing *B. pluton*
40,000	3	2	12	11
4,000	3	2	12	10
400	3	0	12	1
Controls (no *B. pluton*)	3	0	12	0

Most of the larvae which were infected had very large numbers of *B. pluton* organisms in their digestive tracts, and examination of the colony 3 weeks after inoculation showed that European foul brood had spread rapidly, a very large proportion of the larvae being affected.

Exp. 2. A suspension containing approximately 23×10^6 *B. pluton* organisms per ml. of Tyrode solution was prepared. 0·5 ml. of this suspension was injected through the chorioallantoin membrane into a 6-day embryo, and this was apparently sterile after 5 days' incubation and still alive. 1 ml. produced no growth when inoculated into 15 ml. of liquid beef digest brood filtrate agar which was allowed to solidify and then incubated.

On 13 July three different nuclei were selected and fifty young larvae about 1–2 days old in each were fed 0·001 ml. portions of the above suspension, or dilutions prepared from it, so that individual larvae received either 23,000, 2300 or 230 cells of *B. pluton*. Three days after inoculation ten of the inoculated larvae were removed at random from each nucleus and were examined microscopically. Seven of those which had received approximately 23,000 *B. pluton* organisms were affected with European foul brood, three of those which had received 2300 bacteria were similarly affected, but none of those fed 230 organisms had contracted the disease. Subsequent examinations showed that European foul brood spread rapidly in the two nuclei in which the larvae had been most heavily inoculated but did not occur during the remainder of the brood rearing season in the other nucleus.

The above experiments show that young larvae readily contract European foul brood when inoculated with relatively small doses of *B. pluton* organisms which are unable to produce growth on embryo medium or on beef digest brood filtrate agar.

(iii) *The development of* B. pluton *in the bee larva*

White (1920) stated that "Growth of *B. pluton* in the infected larva begins close to the surface of the peritrophic membrane in contact with the food of the larva. As growth continues the mass extends towards the lumen of the peritrophic sac finally filling it more or less completely. The growth does not always take place uniformly along the peritrophic membrane, nor does it extend beyond it, but is enclosed within the sac, the tissues of the larvae not being reached. The multiplication of the organism after the death of the host, if, indeed, it takes place at all, is limited." Morgenthaler (1930) has verified these statements, at least in part, and similar results have been obtained by the writer. These are given below.

Larvae of all different ages and stages of European foul brood were removed from affected bee colonies. They were immediately fixed in Carnoy's fluid, later imbedded in paraffin wax and sections cut. The sections were stained with Ehrlich's haematoxylin, Gram's stain and eosin. It was found that the tissues stained well by haemotoxylin or

eosin, while the *B. pluton* organisms, being Gram positive, were best stained by this stain. A detailed description of the photomicrographs prepared from these sections (Pls. XXXIII–XXXV, figs. 1–17), is appended. These photographs show the distribution of *B. pluton* in its host at different stages in the disease.

DISCUSSION

The fact that *B. pluton* has refused to grow on any medium other than that afforded by the food mass within the peritrophic sac of the developing bee larva indicates that this organism is a strict parasite. Previous attempts to cultivate this species in starved larvae (Tarr, 1937) also met with failure, but it is possible that it will develop in larvae reared in the laboratory by a method similar to that described by von Rhein (1933). Trager (1936) claims that he has grown the virus of Grasserie in tissue cultures of silkworm ovaries, and it would seem worth while attempting to cultivate *B. pluton* on tissue cultures of bee larvae. It may prove impossible to grow this species, but further attempts should be made. It is of interest that relatively small numbers of *B. pluton* organisms are able to initiate European foul brood in young larvae when mixed with the food mass surrounding them. In this respect European foul brood differs radically from American foul brood, where inoculation of individual larvae with large doses of the causal organism has always failed to produce the disease (Tarr, 1938*b*). It has been shown that European foul brood is purely a disease of the digestive tract of the larva, *B. pluton* growing only in the food mass within the peritrophic membrane and probably killing its host before it, or any of the other bacteria associated with the disease, succeed in invading the body tissues. Whether this also applies to larvae affected with American foul brood remains to be determined.

SUMMARY

B. pluton will not grow on the chorioallantoin membrane of the developing chicken embryo or on embryo brei medium made from minced embryos.

European foul brood is a purely intestinal infection of the bee larva, the causal organism being localized in the food mass within the peritrophic membrane.

Young larvae are readily infected with European foul brood by mixing relatively small numbers of *B. pluton* organisms, obtained from recently affected larvae, with the brood food surrounding them.

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EXPLANATION OF PLATES XXXIII–XXXV

The following symbols have been used, most of which are the same as those employed by Nelson (1924). *An*, Anus. *B.m.*, Bacterial mass. (Food matter plus bacteria in badly affected larvae.) *B.p.*, *Bacillus pluton*. *M.Int.*, Mid intestine. *Mth*, Mouth. *Oe.*, Oesophagus. *P.gr.*, Pollen grain. *P.mb.*, Peritrophic membrane. *qg.*, Food matter in mid intestine. *Slk.Gl.*, Silk gland. *Str.Bor.*, Striated border of epithelium of mid intestine.

PLATE XXXIII

Fig. 1. ×37·5. Transverse section through a larva 2–3 days old recently affected with European foul brood. *B. pluton* organisms occur only between the peritrophic membrane and food matter. The uneven distribution of *B. pluton* along the peritrophic membrane is shown by the fact that this organism was present in only a few of the sections made from this larva. The peculiar "granular" structure of the so-called "peritrophic membrane" was described by Nelson (1924) and can be seen in figs. 1–9. It is not known whether the membrane alters when the larvae are "weaned" from brood food to honey and pollen, nor whether it is of chitinous nature, as in adult bees and many other insects.

Fig. 2. ×37·5. Transverse section through another portion of the larva used in making Fig. 1, only showing somewhat heavier invasion by *B. pluton*. This figure also serves to show the uneven distribution of *B. pluton*.

Fig. 3. ×60. A portion of Fig. 2, somewhat enlarged, showing the clear line of demarcation between the *B. pluton* organisms and the peritrophic membrane.

Fig. 4. ×600. Part of Fig. 3, greatly enlarged, showing the penetration of *B. pluton* organisms into the food mass but not into the peritrophic membrane.

Fig. 5. ×12. Larva about 3 days old, at the time of weaning from brood food to honey and pollen, recently affected with European foul brood. The longitudinal section shows the sharp demarcation between peritrophic membrane and food mass as well as the irregularly distributed groups of *B. pluton* between these.

Fig. 6. ×60. An enlarged portion of the longitudinal section used in making Fig. 5. Groups of *B. pluton* are shown between the food matter and peritrophic membrane.

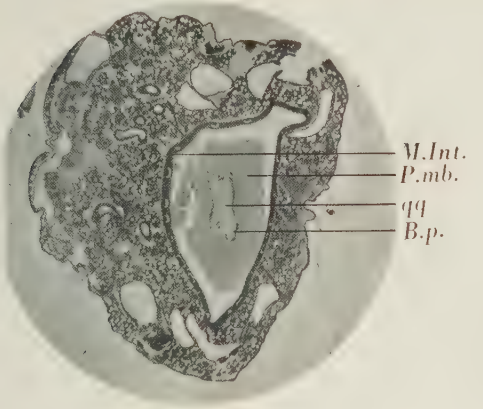


Fig. 1.

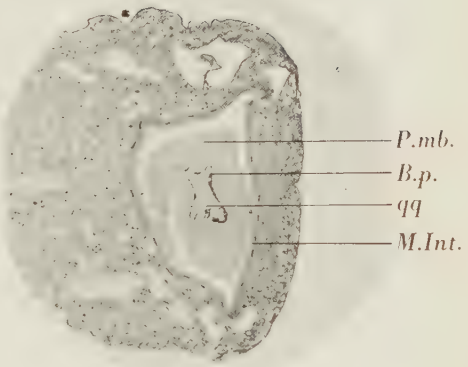
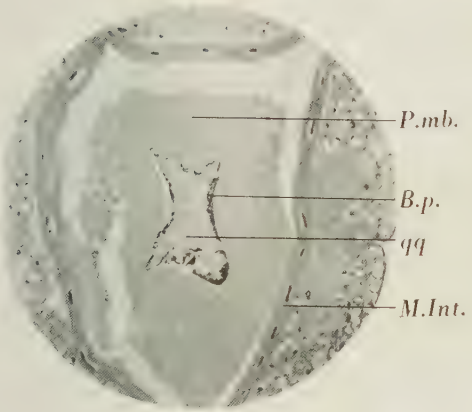


Fig. 2.



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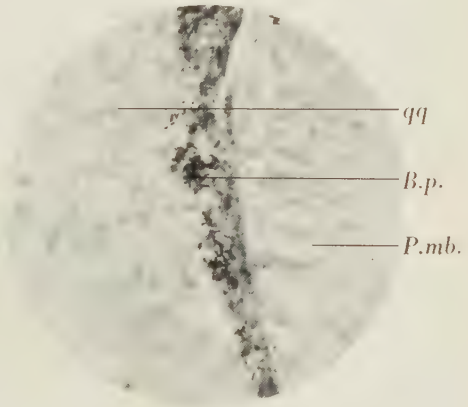

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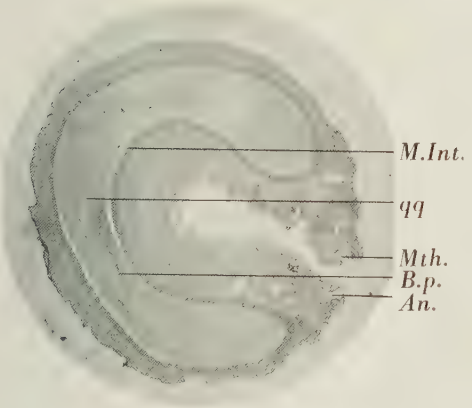

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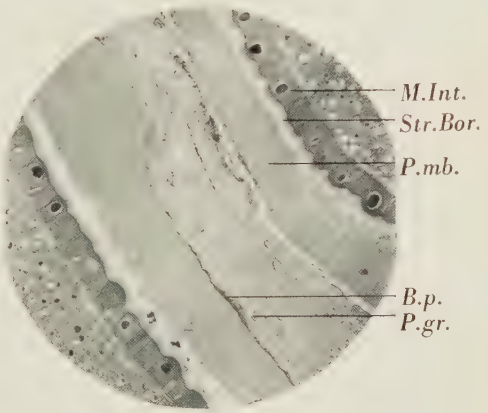

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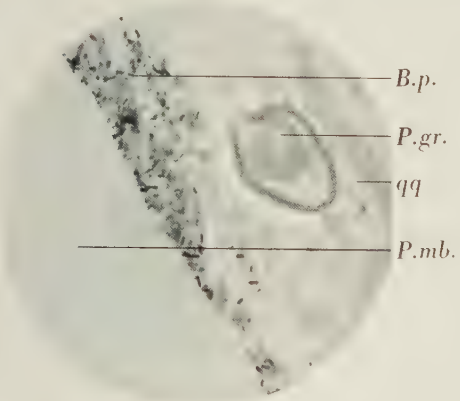

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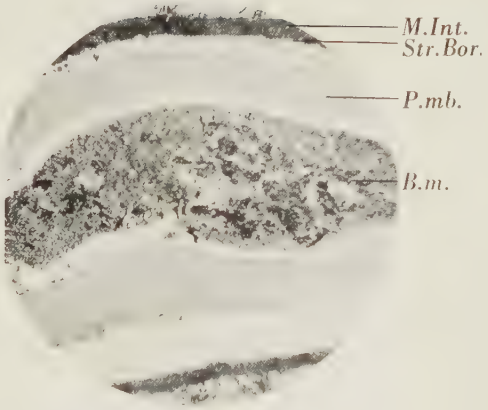

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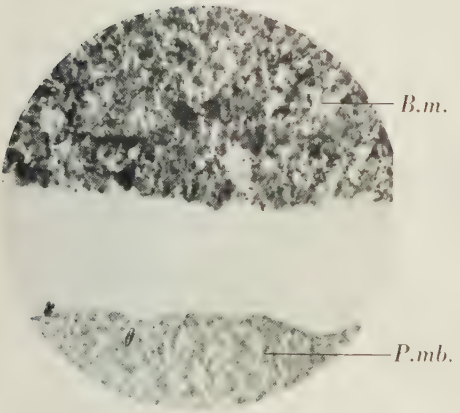

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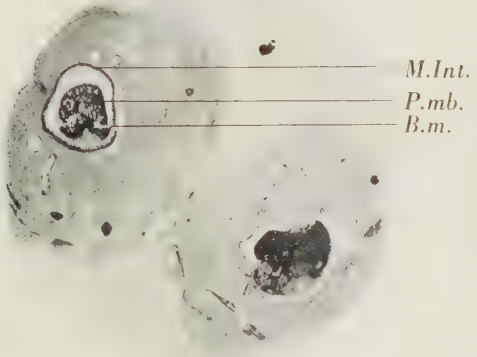

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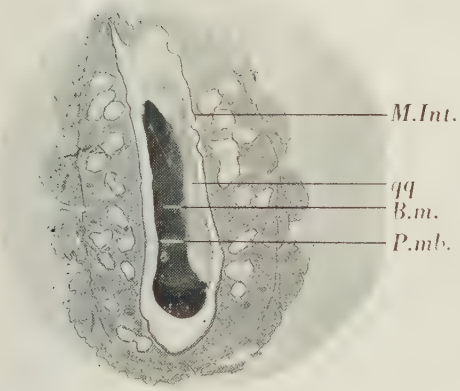

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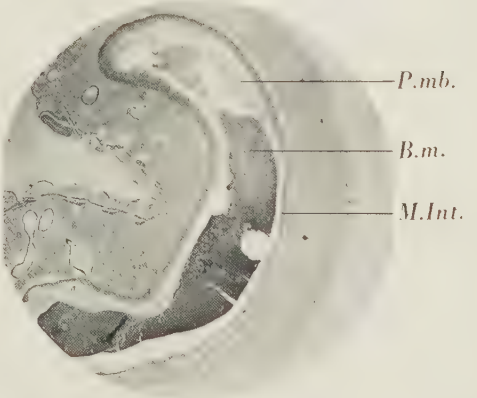
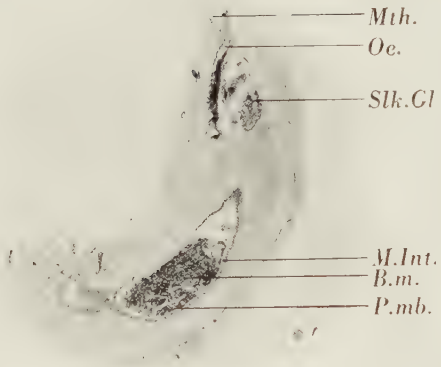


Fig. 12.

Fig. 13.



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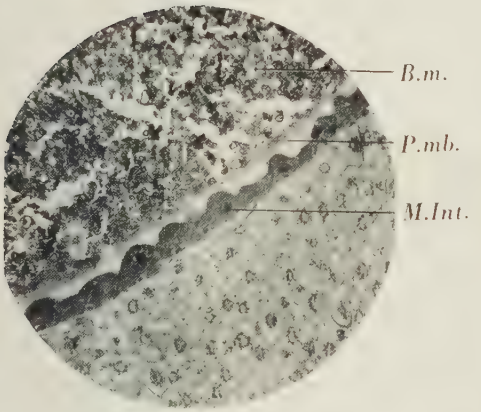

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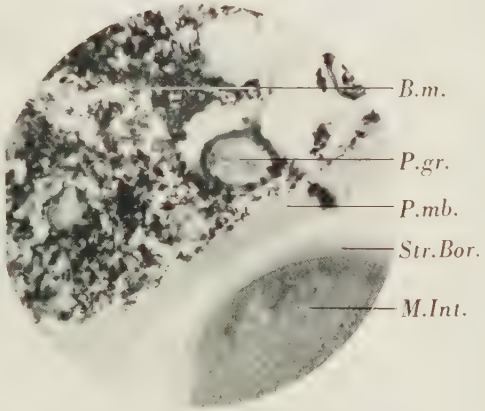

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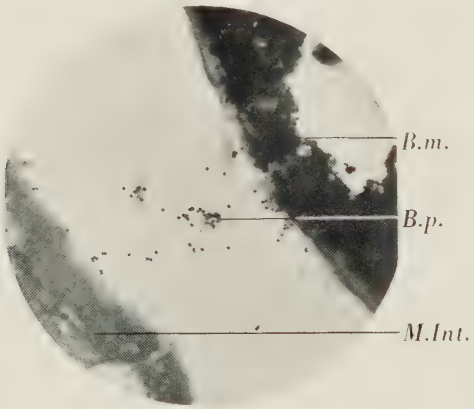

Fig. 17.

PLATE XXXIV

Fig. 7. ×600. A greatly enlarged portion from the section used in making Fig. 6. *B. pluton* is shown penetrating into the food matter but not into the peritrophic membrane.

Fig. 8. ×60. Transverse section of a larva from 3 to 4 days old in which European foul brood has reached a fairly advanced stage. The whole food mass has been penetrated by *B. pluton*, but the peritrophic membrane is still apparently intact.

Fig. 9. ×600. Enlarged portion of the section used in making Fig. 8. The whole food matter has been invaded by *B. pluton* while the peritrophic membrane is still sharply demarcated from the infected area.

Fig. 10. ×12. A transverse section through a coiled larva about 4 days old very badly infected with European foul brood and probably dead. The whole mass of food has become practically a sac of bacteria, but these have not yet penetrated the body cavity of the larva. The peritrophic membrane has nearly disappeared. The epithelium of the intestine has apparently disintegrated.

Fig. 11. ×12. A semi-transverse section through the larva used in making Fig. 10. The bacteria are not evenly distributed through the food mass. The peritrophic membrane has nearly disappeared and the epithelium of the mid intestine has disintegrated.

Fig. 12. ×12. Longitudinal section through a 4–5 day old larva badly affected with European foul brood and probably dead. The whole food mass is full of bacteria, the peritrophic membrane has practically disintegrated, and the intestinal epithelium has lost its characteristic structure.

PLATE XXXV

Fig. 13. ×7·5. A larva about 5 days old very badly affected with European foul brood and stretched out ready for sealing. The food mass has been entirely filled with bacteria and has sunk to form a bacterial sac at the rectal end of the larva. The peritrophic membrane has almost disappeared, and the intestinal epithelium is disintegrating. An affected silk gland is shown; the causal organism evidently penetrates this gland via the opening into the oesophagus.

Fig. 14. ×7·5. A section similar to that used in making Fig. 13 and from the same larva. The oesophagus and silk glands have been invaded by *B. pluton*.

Fig. 15. ×60. An enlarged portion from Fig. 14 showing the food mass invaded by bacteria and full of pollen grains. The peritrophic membrane has practically disappeared, and the mid intestinal epithelium is disintegrating.

Fig. 16. ×600. A portion of Fig. 15 greatly enlarged. The food mass has been entirely invaded by *B. pluton* and other bacteria (secondary invaders), and only a thin layer of peritrophic membrane remains, but this still prevents the bacteria from penetrating the intestinal epithelium.

Fig. 17. ×600. A section through a 4–5 day old larva very badly affected with European foul brood. *B. pluton* and secondary invaders are seen penetrating the remains of the peritrophic membrane preparatory to invading the body cavity of the larva, which is probably dead.

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ON THE CHANGES IN CHEMICAL COMPOSITION ASSOCIATED WITH LARVAL DEVELOPMENT IN THE SHEEP BLOWFLY

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From the Department of Entomology, London School of Hygiene and Tropical Medicine

(With 5 Text-figures)

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Introduction

While the study of the biochemical changes associated with insect development has attracted an increasing number of workers during recent years, most attention has been paid to changes taking place during metamorphosis, and those associated with larval development, though of greater interest in the study of growth in general, have been somewhat neglected. The paucity of chemical information on the early larval stages of insects is, probably, partly due to the difficulties of obtaining and handling the necessary amounts of material; for example, in the course of the present work some 28,000 newly hatched larvae were used in two fatty acid estimations alone.

The sheep blowfly, *Lucilia sericata* Mg. (Diptera, Muscidae), suggested itself as a subject by reason of its short life-cycle, and the relative ease with which a permanent culture can be maintained for the supply of the necessary numbers of eggs throughout the year; it has the additional advantage of having been the subject of a considerable amount of work

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on related problems, such as the nutritional requirements of the larvae (Hobson, 1933, 1935*a* and *b*), and the chemical changes during metamorphosis (Evans, 1932). Muscid larvae were also the subject of Weinland's classical work on the synthesis of fat from protein (1909), which, despite the work of later investigators (Nishikata, 1922; Yuill & Craig, 1937), has still to be confirmed with sterile material and using satisfactory analytical methods.

Material and methods

Egg material for analytical work was used within 1–3 hours of oviposition, to avoid discrepancies due to the rapid embryonic development. Newly hatched larvae were obtained by keeping eggs in saturated air at 25° C. for about 18 hours; the larvae were allowed to wander from the eggs on to filter paper, from which they could readily be collected on the point of a seeker. Their inability to resist desiccation renders rapid handling essential, and their removal in sufficient quantity direct from a glass surface was found impracticable. For later stages the larvae were reared on minced lean beef at 25° C. Body weight was found to be a better measure of state of development than age, and mean weights were determined, either for each whole batch or, with smaller larvae, for half a dozen or so samples of 20–50 larvae each. When full grown the larvae were allowed to migrate into clean dry sand; "prepupae" were used within a day of leaving the meat, after the crop had been emptied. All stages except the unfed newly hatched larvae were washed with several changes of distilled water and dried on filter paper before use; this treatment was found to be without effect on the moisture content.

Total fatty acids and unsaponifiable matter were estimated by a saponification method, as used by Evans (1932, 1934); mean molecular weight determinations, however, showed his second saponification to be unnecessary if the number of washings is adequate. Iodine values were determined by the method of Rosenmund & Kuhnhenn (1923). Total nitrogen was estimated by the micro-Kjeldahl method, which could be used for individual full-grown larvae. Total glucose, after acid hydrolysis, was determined by the Hagedorn-Jensen method, zinc hydroxide being used as a protein precipitant.

Results and discussion

On account of the 700-fold increase in weight during larval development the percentage of each constituent was considered the most useful figure for plotting. The best measure of the state of development of the larva was considered to be provided by body weight, which was plotted logarithmically, since for a considerable part of development there is a linear relationship between log. weight and age (unpublished results).

The limits of the three larval instars are indicated by arrows, and a rough time-scale for the temperature used (25° C.) is also given with each figure. Each point represents a single determination; the crosses apply to larvae, while the circles to the right of each curve apply to the first

day of the prepupal stage, and corresponding values for the newly laid egg are given for comparison by the circles on the left.

(i) *Moisture*

The newly laid eggs contain about 79% water, distinctly less than the larvae at hatching (83%) (Fig. 1); this is probably due to the low moisture content of the egg-shell. From hatching until the middle of the third instar the moisture content falls gradually to about 80%, and then shows a very marked decrease to just under 70% in the prepupae. Progressive dehydration is of very general occurrence in growing organisms, and the

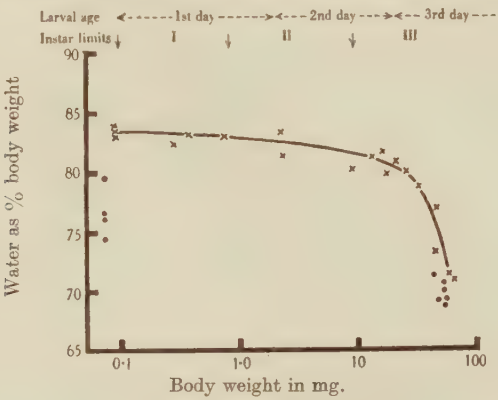

Fig. 1. Changes in moisture content.

accompanying decrease in the extent of dispersion and hydration of the tissue proteins is considered likely to be one factor in the usual decline in metabolic rate with age (Jordan Lloyd, 1932). There is certainly a considerable similarity between the present moisture content results and those found for respiratory rate (to be reported in detail in a later paper); the oxygen uptake falls only from 7 c.c./g./hr. at hatching to about 5·5 c.c./g./hr. at the end of the second instar, while during the third the rate falls to 2 c.c./g./hr. in the full-grown larva and less than 1 in the prepupa. However, the accumulation of inert reserve material during the third instar is likely to be another factor concerned in the declining metabolic rate.

The final rapid fall in moisture content is probably associated with the fact that the whole of larval life is spent in the saturated environment provided by the food, while after feeding ceases the larva leaves the meat and migrates to the much drier surroundings to pupate. The change is

not, however, passive desiccation, since it certainly begins before the meat is quitted. Furthermore, a decrease in moisture content has been found in a number of other insects to accompany an increase in resistance to low temperatures (Buxton, 1932), and the prepupa is the stage in which this species overwinters.

Tangl (1909) gave a moisture content of 69% for larvae of *Ophyra*, the corpse fly, probably full grown, and Tomita & Kumon (1936) recorded 77% for 15 mg. larvae (probably early third instar) of the flesh-fly *Sarcophaga*. Yuill & Craig (1937) give some rather variable figures for *Lucilia sericata* showing a fall from about 83% at the end of the second instar to about 70% in the full-grown larvae. These data all agree substantially with the present results, but Rudolfs' figures (1926*a*) for the tent caterpillar *Malacosoma* differ considerably, perhaps associated with the more exposed environment of the leaf-eating caterpillars; they showed an increase from less than 40% at hatching to 84% during the latter part of larval life, followed however by a fall before pupation as in *Lucilia*. Larvae of the wax-moth, *Galleria*, living in the probably more humid atmosphere of the bee-hive, behave more like *Lucilia*, showing a fall from 75% water at 10 mg. body weight to 56% at 200 mg. (Teissier, 1931).

(ii) *Fats*

The eggs when laid have a fatty acid content of 17–19% dry weight, falling to 9–13% in the newly hatched larvae. From these figures, together with further evidence from work on the respiration of the eggs, to be published in a later paper, fat appears to be the main energy source during embryonic development. This is also the case in such other insects as have been investigated—the silkworm *Bombyx* (see Needham, 1931), the tent-caterpillar, *Malacosoma* (Rudolfs, 1929), and the grasshopper *Melanoplus* (Slifer, 1930; Boell, 1935)—and would of course be expected from the fact that the class as a whole produces typical cleidoic eggs.

The proportion of fat probably shows little change till the end of the second instar, when a rapid accumulation begins, continuing to the end of larval life, when a maximum of nearly 30% is reached, falling to about 27% within the first day of leaving the meat (Fig. 2). This agrees closely with the conclusions of Davies & Hobson (1935); they demonstrated a rapid increase in the resistance of the larvae to starvation (in saturated air) between the early second and early third instars, and suggested that the storage of reserve material probably began at about this stage. There are a number of records of increasing fat content during larval life, e.g.

Rudolfs (1926*a*) showed an increase from 1% at hatching to 29% at pupation in *Malacosoma*, and Teissier (1931) found the fat content of the meal-worm *Tenebrio* to increase as the 1·13th power of the body weight during the latter part of larval life.

In nearly all such work, however, fat has been estimated as crude ether extract; a Soxhlet extraction of the prepupae was carried out for comparison with the present saponification results. 22·5% crude fat was obtained, corresponding to 20% fatty acids, as compared with the 25–27·5% obtained directly. Discrepancies in the earlier stages are likely to

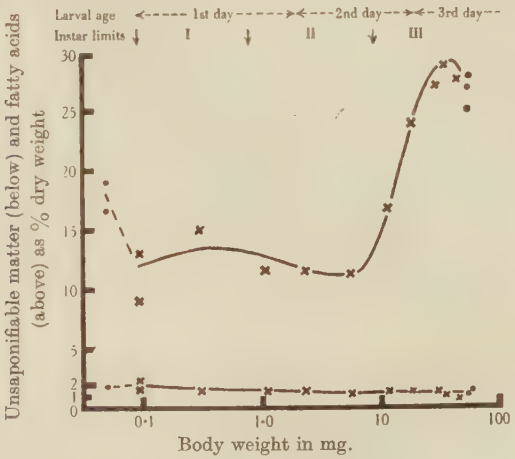

Fig. 2. Changes in fats.

be considerably greater on account of the smaller size of the larvae, the smaller proportion of fat present, and the fact that much of the vital fat is probably in the form of complex lipoids not readily extracted with ether. This point is illustrated by the recently published work of Yuill & Craig (1937), on the larval fat metabolism in this species. They found 6% fat (crude ether extract) in the earliest stage studied (late second instar), while the present results give 14% (glycerides plus unsaponifiable) at this stage. The discrepancy decreases as the fat content rises; Yuill & Craig find a maximum of 30%, while the saponification method gives one of 31%. The data of these authors confirm the rapid storage of fat during the third instar which was found in the present investigation, but it is unfortunate that out of their thirty-seven fat estimations on larvae, none was done on first instar material, three during the second, and all the remainder during the third.

The 11% or so of fatty acids present during the first two instars in this material is probably mainly "élément constant" or "vital fat", though some of it may represent hydroxy-fatty acids produced by the deamination of the food proteins, which are possibly the larva's main energy source (Brown, 1936). Evans (1932) found an acetyl value of zero during metamorphosis, indicating an absence of hydroxy-acids at that time, but no information is available for the larval period. It is also possible that the very high metabolic rate of the larvae, presumably involving great activity at cell surface membranes, may be associated with a relatively high "élément constant".

With two exceptions, all the iodine values obtained for larvae lie between 67 and 73, and the first exception, a low value obtained on a very small sample of fatty acids from newly hatched larvae, seems almost certainly aberrant. In the case of the second, however, a value of 92 was obtained at the beginning of the third instar; duplicate determinations on the same batch of fatty acids differed by less than one unit, and so this value may possibly be significant, particularly as it applies to the stage when the accumulation of reserve fat is just beginning. It therefore seems likely that the degree of unsaturation of the fatty acids does not vary appreciably during larval life, with the possible exception of an increase when the accumulation of reserve fat begins. During the latter half of larval life Yuill & Craig also found a steady iodine value, with the exception of a slight fall near the end on the fish diet (possibly representing an increase in the proportion of fat synthesized from protein to fat derived directly from the food, in the reserves being laid down). These results do not agree with the usual idea of a highly unsaturated "élément constant", and further evidence that this idea is not applicable to *Lucilia* is provided by Evans's observation that during metamorphosis the saturated acids remain practically constant, only the unsaturated ones being consumed. The possible significance of the low iodine values found for the eggs (34–44) is discussed in a later section.

Another factor likely to influence the degree of unsaturation of the reserve fat is the environmental temperature; similar cultures were set up at 15, 25 and 35° C., and batches of prepupae were saponified within 24 hours of leaving the food in each case. Iodine values of 75·8, 72·7 and 71·0 were recorded respectively for the three temperatures, showing differences which are very small compared with those found, e.g. by Terroine *et al.* (1927), for certain fungi and bacteria; further, even the differences observed may be explained in part by the selective utilization of unsaturated fatty acids (Evans, 1932), which begins on leaving the

meat, and which will certainly occur at a rate varying with the temperature.

Yuill & Craig, however, have demonstrated the large qualitative effect of the fat in the diet on the reserve fat laid down; on a fish diet containing an oil of iodine value 113, the larval fat gave an iodine value of 120–140, while on a diet containing butter as the source of fat (I.V. 30), the iodine value of the larval fat was 60. These results suggest that while, as Weinland (1909) showed, the bulk of the reserve fat is probably synthesized from protein, yet preformed fat in the diet is also incorporated in the larval reserves.

The evidence so far available thus suggests that of the various factors likely to influence the degree of unsaturation of the fat in the larva of *Lucilia*, the nature of the food fat is easily the most important; the state of development appears to have no effect, except for the possible rise at the beginning of the third instar; and, at any rate on a diet containing preformed fat (such as is likely to be encountered in nature), the effect of environmental temperature is very slight.

Samples of fatty acids from the prepupae gave mean molecular weights of 272–290, in satisfactory agreement with Evans's figures of 270–287. Both iodine value determinations for this stage gave 72·7 (at 25° C.); Evans found 79–96 at 17° C.

Though the figures for unsaponifiable matter should not perhaps be taken too seriously, on account of the very small amounts of material concerned (usually less than 10 mg.), the general trend of the figures indicates a fall, from about 2% at hatching to about 1% in the prepupa. This fraction is of particular interest in view of Hobson's demonstration (1935) that cholesterol is the only fat-soluble substance essential for the larval growth of *Lucilia*.

(iii) *Ash*

As pointed out by Teissier (1931), the ash may often be considered as a very rough measure of the protoplasm present, consisting as it does mainly of the mineral salts which form an essential constituent of living matter; reserve materials and chitin are usually practically ash-free. In some cases this generalization is inapplicable on account of the large amounts of inert skeletal or excretory material such as calcium salts and silica, but the results of Tomita & Kumon (1936) for larvae of another blowfly, *Sarcophaga*, showing that 84% of the total ash consists of the phosphates and chlorides of sodium and potassium, suggest that it may safely be applied here.

The newly laid egg contains about 7% ash, while the larva has about 10% at hatching; the observed consumption of fat during embryonic development only accounts for about one-fifth of this difference, suggesting a low ash content for the egg-shell.

The percentage of ash falls steadily during larval life, most rapidly during the latter part of the third instar (Fig. 3). In part this is explained by the storage of fat, but this is not the entire and probably not even the chief cause, for when expressed in terms of fat-free dry matter ash still falls from more than 11% at hatching to 5% in the prepupa. This demonstrates a considerable accumulation of materials of low ash content in addition to fat, and there is other evidence, discussed later, that much of this may be reserve protein.

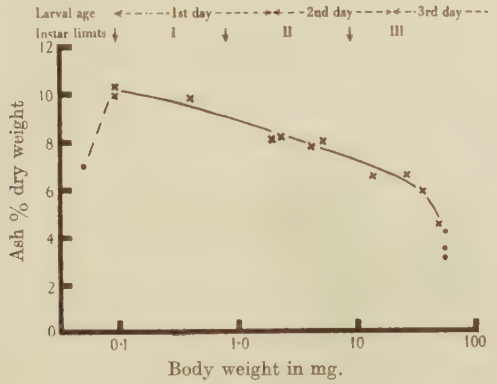

Fig. 3. Changes in ash.

Tomita & Kumon recorded 7% of ash for *Sarcophaga* larvae at about the beginning of the third instar, in close agreement with the present results; Teissier also found a considerable decrease in ash during larval life, from 5% at 10 mg. to 1% at 200 mg. body weight in *Galleria*.

(iv) *Nitrogen*

The results of Evans (1932) for this species show that 82% of the total nitrogen is in the form of protein at the beginning of the prepupal period; furthermore Brown (1936) has shown that the total ammonia excreted during larval life in *Calliphora* on a meat diet only amounts to about 2·3% of the body weight at pupation, and hence the amount of ammonia present at any given period will be small compared with the amount of body protein. It does not, therefore, seem unreasonable to

make the usual assumption of regarding the total nitrogen of the larva as a rough measure of the protein present.

The eggs gave a weighted mean of 12·5% nitrogen and the newly hatched larvae one of 12·7%, while the egg-shell material left after hatching gave 11·4–11·6%. The chorion gave strongly positive Millon and xanthoproteic reactions, but the nitrogen content found indicates non-protein constituents as well. The newly laid egg was completely soluble in potash under the conditions prescribed by Campbell (1929), indicating the absence of chitin at this stage, though the cuticle of the newly hatched larvae gave a strongly positive van Wisselingh reaction.

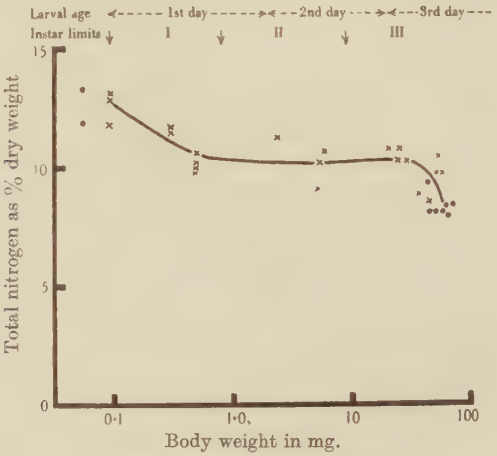

Fig. 4. Changes in nitrogen

Molisch's test, though positive for the contents of the undeveloped egg, was completely negative for the chorion, showing the absence of other polysaccharides and of mucoproteins. Another possibility is the presence of fats or waxes in the shell; the loss of fatty acids calculated from the mean values found for the newly laid egg and the newly hatched larva is actually about 10% greater than can be accounted for by the whole of the oxygen uptake during embryonic development, and, while on account of the variability of the analytical data this fact is in itself of little significance, yet, taking into account also the low iodine values found for the eggs, the evidence available seems to point to the presence of saturated fatty acids in addition to protein in the chorion.

The nitrogen content of the larvae falls from about 12·5% at hatching to about 10% by the end of the first instar (Fig. 4), and appears to remain

at about that figure till practically the end of larval life, when a further fall occurs, to about 8·5% in the prepupa; the latter change will represent mainly the nitrogen excreted after feeding ceases. Similarly in *Malacosoma* Rudolfs found a fall from 15% at hatching to a minimum of 8·5% in late larval life. Owing, however, to the considerable changes which are taking place in other constituents, notably fat, Table I, constructed from the present results, is perhaps more instructive than the percentage figures.

Table I. *Nitrogen/ash ratios of larvae at various stages*

Stage	Body weight in mg.	Nitrogen/ash
Hatching	0·09	1·3
Mid 1st instar	0·25	1·2
End 1st instar	0·80	1·1
Mid 2nd instar	3	1·3
End 2nd instar	9	1·4
Early 3rd instar	20	1·5
Late 3rd instar	40	1·7
Prepupa	55	2·3

It has previously been pointed out that most of the ash probably represents the mineral salts which are an essential constituent of protoplasm, and that the greater part of the nitrogen is present as protein. Since the composition of protoplasm is unlikely to vary to the extent shown by Table I, it is suggested that at the end of larval life something approaching half the protein present represents relatively ash-free reserve material. There is some histological evidence also for this storage of protein; the cells of the pupal fat-body contain numerous semi-solid granules giving positive biuret and xanthoproteic reactions (Evans, 1935). It seems likely that these will have been formed by precipitation at their isoelectric point, and hence that their ash content will be at a minimum.

Table I also suggests the disappearance of a certain amount of reserve protein during the first instar; reserves of some sort must almost certainly be present even at hatching, since under saturated conditions the unfed larvae can survive for 21 hours even at 37° C. (Davies & Hobson, 1935). Some utilization of reserves will probably occur normally while the larvae are establishing themselves on the meat.

As the food of the larvae consists very largely of protein, it would be expected that the error, present in all these experiments, due to the presence of undigested food in the crop, would be most serious in the nitrogen estimations. Three determinations of the nitrogen content of gorged full-grown larvae gave a mean of 2·64% (fresh weight), as compared with 2·49% after the crop was emptied. It is, therefore, probable that the

whole of the curve given, with the exception of the points for the egg, the newly hatched larva, and the prepupa, is something like 10% too high; this correction would accentuate the change in the nitrogen/ash ratio recorded above.

The presence of a variable amount of food protein in the crop is probably also partly the cause of the nitrogen figures showing more variability than the other determinations, though the small size of the samples used will also give greater prominence to individual variation.

(v) *Carbohydrate*

As a contrast to the results obtained for fats, the egg was found to contain a slightly lower percentage of carbohydrate than the newly hatched larva. During larval life the percentage of total glucose falls from about 10 to 7% (Fig. 5). If, however, fat is deducted and a similar

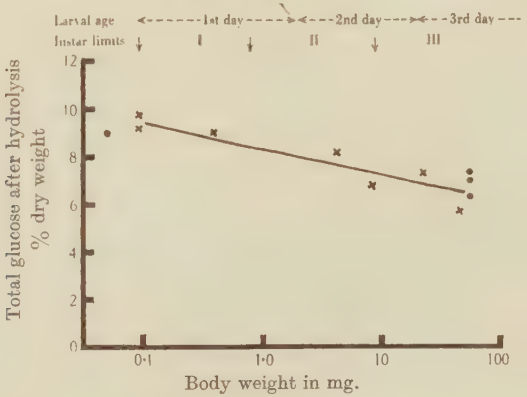

Fig. 5. Changes in carbohydrate

rough correction, based on the nitrogen/ash figures, applied for reserve protein, glucose as a percentage of the remaining dry matter falls from 12 to 9·5% during the first instar, remains at about 9% during the second, and finally increases to 14% in the prepupa. If the validity of this treatment of the results be accepted, it would appear that reserve carbohydrate is utilized in addition to protein, while the larvae are becoming established on their food medium, and that a further reserve of carbohydrate is laid down during the third instar.

The recent work of Kuwana (1937), on the body fluid of *Bombyx*, suggests however that some caution is necessary in the interpretation of results of this kind, particularly in expressing reducing power in terms of

glucose; for example, the amount of glucose present in the body fluid of the silkworm as free fermentable reducing agent is negligible, except for a temporary rise after feeding to about one-eighth of the total reducing power of the deproteinized filtrate. Acid hydrolysis, however, roughly doubles the total reducing power; practically the whole of this increase was shown to be fermentable, and at least half of it could be attributed with certainty to glycogen. Kuwana followed the total reducing power, both before and after hydrolysis, from the beginning of the fourth instar; in both cases maxima were obtained just before the fourth moult and just before pupation. Glycogen, estimated directly, remained at a steady value during the larval stages and rose to a maximum of about 2·5 times this value at pupation.

Considering glycogen alone, Rudolfs found a fall from 2·8 to 0·2% during larval life in *Malacosoma*, the actual amount present, however, increasing some thirteen times over this period. In the moth *Dictyoploca* water-soluble reducing sugars increased during the first few days of larval life, but decreased rapidly thereafter; glycogen also was only found in traces in the pupa (Shinoda, 1926). In the bot-fly *Gastrophilus* much glycogen is formed in early life, but a large proportion is later transformed to fat (Kemnitz, 1914; Blanchard & Dinulescu, 1932). The fully grown bee larva on the other hand contains considerably more glycogen than fat (Straus, 1911).

Summary

The most marked change in composition during embryonic development is the disappearance of fat typical of cleidoic eggs.

The chorion probably consists of protein together with some nitrogen-poor constituent, perhaps a saturated fat or wax; chitin and other carbohydrates are absent, and ash and moisture contents are low.

No great changes in composition occur during the first two larval instars, with the exception of a possible utilization of some reserve protein and carbohydrate while the larvae are establishing themselves on the meat.

During the third and last instar, however, a considerable reduction in moisture content occurs, and an extensive storage of fat takes place, beginning at about the stage at which previous work had shown a marked increase in resistance to starvation. There is also evidence for the accumulation of a large bulk of reserve protein and a smaller amount of reserve carbohydrate during this instar.

The most important factor influencing the degree of unsaturation of the larval fat appears to be the nature of the food fat; iodine values show little variation with the state of development, and preliminary observations suggest that the influence of the environmental temperature is small.

I wish to record my sincere thanks to Prof. P. A. Buxton for his active interest in this work, and to Dr V. B. Wigglesworth for helpful advice and valuable suggestions. I am also indebted to the Agricultural Research Council for the award of a Research Scholarship which has entirely financed this investigation.

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THE USE OF PROTECTIVE FILMS OF INSECTICIDE IN THE CONTROL OF INDOOR INSECTS, WITH SPECIAL REFERENCE TO *PLODIA INTERPUNCTELLA* Hb. AND *EPHESTIA ELUTELLA* Hb.

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Introduction

Insects belonging to the genera *Ephestia* and *Plodia* are among the principal pests of stored products in this country and elsewhere. The two most important species are probably *Ephestia elutella* Hb., the cacao moth, which is one of the chief pests of cacao, dried fruits and tobacco, and *Plodia interpunctella* Hb., the Indian meal moth, which is a very important pest of dried fruits, nuts and cereals.

One of the noteworthy points in the biology of these two species is that the majority of the fully grown larvae leave their food and wander away to pupate in some crack or crevice. In this way a large number

reach the fabric of the warehouse storing the infested goods. The moths which result from these larvae will infest any susceptible goods that are in the warehouse when they emerge.

It is evident that for the purpose of control there are two sources of infestation. The first is the insects which arrive from outside the warehouse, either by way of infested goods or by other means; the second is from inside the warehouse, due to the moths emerging from the premises.

With a few possible exceptions, goods can be freed from infestation prior to storage, by fumigation in suitable chambers or by other means. This treatment will ensure that no infestation is imported with the goods. The clean goods can then be taken to the warehouse. The problem of protecting the clean goods from reinfestation by insects from the warehouse or elsewhere, is more difficult.

One method of cleaning the warehouse is to leave it empty throughout the summer months, so that there is no food upon which moths emerging from the premises can lay their eggs. Usually this method is not economically possible. A second method is that of fumigating the premises, but unless the warehouse has no cracks and crevices and can be made airtight, it is not certain to give good results. An account of a series of fumigations with hydrogen cyanide and ethylene oxide has been given (Potter, 1937), which shows the difficulty of killing the full grown larvae of *E. elutella* and *P. interpunctella* when they are in crevices in the fabric of the warehouse. The most serious objection to these two methods is that they do not guard against reinfestation during the summer months when the moths are flying. It appears from observation, that infestation by moths which fly in from outside is very likely to occur. A control method was devised to overcome these difficulties. It was essentially a preventive method and consisted of atomizing a solution of pyrethrins in white oil to form a mist in the warehouse. The mist was put up in the warehouse every night throughout the period of moth emergence, so that no moths were able to reach and infest the stored goods. This method was first used for the protection of dried fruits in London, where it was completely successful; the apparatus and technique has been described (Potter, 1935). It was then introduced in Bristol, Liverpool and Glasgow, and warehouses in each of these towns were rid of infestation in a year.

The objection to this method is the expense, since the cost of apparatus, materials and labour to spray every night for about 3 months is heavy.

There were indications during the application of this method that its success was due to a protective film of insecticide which formed on all

exposed surfaces when the mist settled. Any moth settling on this film was incapacitated and finally killed. It was obvious that a study of the film might lead to a modification of the method which would be more economical. The method of forming a protective film of insecticide might also be of use in other branches of industrial and medical entomology.

This paper describes field and laboratory experiments which show that by a modification of the atomizing method previously described, a protective film can be formed which gives complete protection against *P. interpunctella* and *E. elutella* in the warehouse and is also very much more economical in apparatus and labour than the previous method. The experiments also indicate the possibilities of further investigation into the use of protective films of insecticide against other indoor pests.

(*a*) *The objects of the experiments*

(*a*) The production of a film of insecticide in the warehouse which will prevent moths of *E. elutella* and *P. interpunctella* which emerge from the fabric of the warehouse or which enter from outside from infesting the goods stored in the warehouse.

(*b*) A study of the effect of the film on the migrating larvae of these species, so that infestation from this source may be prevented or localized if it does occur.

(*c*) A study of the lasting properties of the film.

(*d*) A short preliminary study of the effect of the insecticidal film on other species of insect.

(*b*) *The insecticide*

The insecticide used throughout the experiments consisted of a solution of approximately 0·8% pyrethrins in a white oil of the following specification: sp.gr. 0·862; flashpoint closed, 320° F., flashpoint open, 335° F.; visc.redw. 1 at 70° F. 118 sec.; unsulphonated residue, 99·2% by vol.; pour test, −30° F., and sufficiently refined to be transparent, colourless and tasteless; this oil which resembles medicinal paraffin is marketed under the name of Shell oil 24210. This insecticide has no fire risk and has already been used on cacao, tobacco and dried fruits without having any deleterious effects.

Previous experiments (Potter, 1935) had shown that this insecticide is effective against the larvae of *E. elutella* and *P. interpunctella* when sprayed directly on to them, and to the adults when atomized in the form of a mist.

In the first three tests in the semi-field scale experiments and in the field scale trial the insecticide was made up by mixing one part of a commercial extract of pyrethrins with seven parts of Shell oil 24210. For the remainder of the tests, an extract of ground flowers with petroleum ether (B.P. below 40° F.) was standardized by the Seil method. This solvent was then removed and the pyrethrins taken up in Shell oil 24210 to make an 0·8% solution. It is thought that the heavy oil used as a carrier makes the formation of a lasting film possible, but no work has been done on the effect of different carriers. Further experiments have shown that other toxic agents, such as the organic thiocyanates, may be used instead of pyrethrins, but there is as yet insufficient knowledge of their effect.

(*c*) *Organization of the work*

The work was first carried out under semi-field conditions, using the commercial apparatus; a full-scale field trial was then made, which proved successful; finally, a more detailed investigation of the properties of the film was made in the laboratory under controlled conditions.

Semi-field experiments

These experiments were carried out at a London warehouse under the normal conditions of unheated storage. A film of insecticide was applied as evenly as possible to boards of Canadian hemlock, an absorbent wood used for making dried-fruit boxes. The film was applied with the atomizing gun which is used in practice and which has already been described and figured (Potter, 1935). The degree of atomization was standardized by timing out a given quantity of the insecticide at a known pressure.

In field experiments of this kind it is very difficult to measure what percentage of the material sprayed on to a surface actually adheres to form a film. Absorbent paper was sprayed under similar conditions to the test boards and, by weighing the paper before and after spraying, it was found that about 0·6 of the material sprayed adhered to form the film.

In the second experiment the box was covered with a glass lid so that the film was exposed to diffused light; in the rest of the experiments the films were kept in the dark. Films of pyrethrum insecticide kept in the dark would last longer than those exposed to light, but in general the interior of a warehouse is dim and would not cause rapid deterioration, and by having a glass lid an uncoated surface was presented which might affect the results of the experiment.

After the boards had been sprayed they were made up into a box with

the coated surfaces forming the interior of the box; the test insects were placed inside. All the experiments were made on fully grown larvae of *P. interpunctella* and *E. elutella*, chiefly on the former since they are more resistant to the insecticide used. The first two experiments were made in the winter and most of the larvae had spun cocoons from which they had to be removed. This treatment made them less resistant than the normal migrating larvae, as is shown by the later experiments, but the difference in resistance does not appear to be very great.

In practice, the essential property of the film is that it should kill the moths, but the larvae are very much more resistant than the moths, and a film which takes a long while to affect the larvae will kill the moths in a very short time. The fully grown larvae were chosen for these experiments because it was possible to obtain them in large numbers, because they are easier to handle and because, in a number of situations, it is desirable that the film should be lethal to the larvae as well as the moths. A preliminary experiment was made to ascertain whether a film of the insecticide could kill the larvae; the interior of a box was heavily coated with insecticide and 150 larvae of *P. interpunctella* and 75 larvae of *E. elutella* put in it. They were severely affected after a short exposure but were left for a week before removal: all the larvae subsequently died.

Having obtained definite evidence that the film could be toxic to the larvae, a further series of experiments was made under more controlled conditions.

The following data give the general conditions of the succeeding experiments: Width of box, 13 in.; length, 20 in.; depth, $6\frac{3}{4}$ in.; giving a total area of 966·5 sq. in. with lid and 705·5 sq. in. without. The distance of the nozzle of the atomizing gun from the sprayed surface was 12 in.–18 in., and the air pressure was 60–70 lb./sq. in. The rate of spraying was 6·8 c.c./min.

The boxes were kept beside a recording bimetallic thermograph and a recording hair hygrometer which were checked each week. In the later experiments the hair hygrometer broke down, so that only temperature records are available. The average temperature and the average relative humidity were calculated by measuring the average height of the curve for the period named.

Experiments 1 *and* 2

The first two experiments were made to determine approximately the amount of insecticide which should be used to form a lethal film, and to obtain some idea of the lasting properties of such a film.

The first box was sprayed with 53 c.c. of the insecticide and the second with 9·4 c.c. The first box was stained with the heavy coating; the amount applied to the second was about as much as is likely to be applied by a single medium spraying in practice.

The insects used in these two experiments were collected from the warehouse; 225 larvae of *P. interpunctella* and 96 larvae of *E. elutella* were put into an unsprayed box as controls. After 6 weeks 150 of the *Plodia* larvae and 66 *Ephestia* larvae were removed and used in the experiments; at the time of removal they were perfectly healthy. The remaining controls all emerged as moths.

The results are set out in Tables I and II and show that, under the given conditions, a film of insecticide of about 0·0053 g./sq. in. is lethal to the fully grown larvae of the two species, and that a film of this weight can retain its toxicity when kept in diffused light, for periods of up to 20 days.

These experiments were done at a time when the larvae are normally hibernating and many of them had to be removed from their cocoons. They were thus more susceptible than larvae under their normal conditions of migration.

A series of experiments based on the foregoing results was made during the summer months to obtain further information on the action of the film. The first experiment of this series was made to determine whether the oil alone could be lethal and the results are given in Table III.

The figures show that very little of the toxicity of the film can be attributed to the oil, because even when the oil was used at a dosage of about 0·029 g./sq. in. it had very little toxicity. It is noteworthy that in one of the series in which the insecticide was used for comparison with the oil film there was 15% survival. The insecticide used in this instance was the commercial extract. The two experiments with the insecticide which gave 100% kill were made with the extract standardized in the laboratory by the Seil acid method.

In the foregoing experiments the larvae were exposed to the action of the film for 7 days in order to eliminate as far as possible the factor of time of exposure, when assessing the properties of the film. In practice, it is desirable that the larvae should be killed after a shorter time of exposure and a series of experiments were made on new and 7-day-old films of approximately 0·0053 g./sq. in. of insecticide to determine how the length of exposure of the insect to the film affected the mortality. The results of this series are given in Table IV.

Table I

First preliminary experiment to test the effectiveness and lasting properties of films of approximately 0·8% pyrethrins I and II in white oil on the fully grown larvae of *P. interpunctella* and *E. elutella*: (*a*) Heavy film. The insecticide was applied to the interior of boxes of Canadian hemlock to produce a film of approximately 0·029 g./sq. in. of insecticide [53 c.c. of insecticide were used]. The larvae were exposed to the action of the film for 7 days. The film was kept in the dark. The season during which the larvae were exposed was from 17 January to 28 February. The total period of the experiment was from 17 January to 18 April.

Test no.	Age of film (days)	No. of larvae		Temperature range during exposure (° F.)			Relative humidity during exposure (%)			Max. period between exposure and death (days)	Temperature range during survival period (° F.)			Relative humidity during survival period (%)			No. surviving to pupate
		P. interpunctella	*E. elutella*	Av.	Max.	Min.	Av.	Max.	Min.		Av.	Max.	Min.	Av.	Max.	Min.	
1	0	150	50	45·5	48·0	44·0	77·4	86·0	72·0	50	45·0	51·0	38·0	74·7	94·0	51·5	0
2	7	200	—	44·8	48·0	41·0	76·8	94·0	66·0	60	45·2	55·5	38·0	72·0	94·0	45·0	0
3	14	200	—	44·0	48·0	40·0	76·0	90·0	53·0	60	45·7	55·5	38·0	71·0	90·0	45·0	1
4	21	100	—	42·0	48·0	38·0	70·0	86·0	51·0	53	45·8	55·5	38·0	70·6	90·0	45·0	0
5	28	100	—	48·0	53·0	46·0	76·4	90·0	58·0	50	46·5	55·5	38·0	69·7	90·0	45·0	0
6	42	100	—	44·0	46·0	42·0	76·0	80·0	65·0	49	46·6	55·5	38·0	66·4	88·0	45·0	0

Table II

Second preliminary experiment to test the effectiveness and lasting properties of films of approximately 0·8% pyrethrins I and II in white oil on the fully grown larvae of *P. interpunctella* and *E. elutella* Hb: (*b*) Light film. The insecticide was applied to the interior of boxes of Canadian hemlock to produce a film of approximately 0·0053 g./sq. in. of insecticide [9·4 c.c. of insecticide used]. The larvae were exposed to the action of the film for 7 days. The film was kept exposed to diffused light. The season during which the larvae were exposed to the film was from 7 March to 27 March. The total period of the experiment was from 7 March to 10 June.

Test no.	Age of film (days)	No. of larvae		Temperature range during exposure (° F.)			Relative humidity during exposure (%)			Max. period between exposure and death (days)	Temperature range during survival period (° F.)			Relative humidity during survival period (%)			No. surviving to pupate
		P. interpunctella	*E. elutella*	Av.	Max.	Min.	Av.	Max.	Min.		Av.	Max.	Min.	Av.	Max.	Min.	
1	0	100	50	40·0	46·0	38·0	61·6	71·0	45·0	61	47·3	60·0	38·0	67·7	88·0	45·0	0
2	7	100	—	46·0	52·0	42·0	72·8	87·0	60·0	54	49·4	60·0	40·0	68·7	88·0	51·5	0
3	20	100	—	49·0	52·0	44·0	63·8	73·0	54·0	81	52·7	62·0	40·0	66·5	88·0	38·0	0

Table III

Experiment to compare the effect of a film of the oil carrier alone with that of similar films containing 0·8% pyrethrins in Shell oil 24210. The films were made on the interior of boxes of Canadian hemlock and were kept in the dark. The test insects were migrating larvae of *P. interpunctella*. Period of exposure to the film was 7 days. The experiments were carried out between 25 June and 6 October 1937.

Insecticide	No. of larvae	Approximate amount of material per sq. in. (g.)	Temperature range during exposure (° F.) Av.	Max.	Min.	No. surviving to pupate	Temperature range during experiment (° F.) Av.	Max.	Min.	% kill
Shell oil 24210	100	0·024	64·0	68·0	60·0	88	64·4	74·0	60·0	12
,,	100	0·024	66·0	68·0	62·0	85	66·6	74·0	62·0	15
,,	100	0·0053	64·0	68·0	60·0	92	64·4	74·0	60·0	8
,,	100	0·0053	66·0	68·0	62·0	97	66·6	74·0	62·0	3
0·8% pyrethrins* in Shell oil 24210	100	0·0053	64·0	68·0	60·0	15	64·4	74·0	60·0	85
,, †	100	0·0053	66·0	68·0	62·0	0	66·6	74·0	62·0	100
,, †	100	0·0053	66·0	68·0	62·0	0	66·6	74·0	62·0	100

* Non-standardized commercial extract.

† Extract standardized by the Seil acid method.

Table IV

Experiment to investigate the effect of varying times of exposure to films of 0·8% pyrethrins in Shell oil 24210 applied at the rate of approximately 0·0053 g./sq. in. to boxes of Canadian hemlock. The films were kept in the dark. The insecticide was made up from an extract standardized by the Seil acid method. The test insects were migrating larvae of *P. interpunctella*. The experiments were carried out between 29 September and 24 November.

No. of larvae used	Age of film	Period of exposure to film	Temperature range during exposure (° F.) Av.	Max.	Min.	No. surviving to spin cocoons	Temperature range during experiment (° F.) Av.	Max.	Min.	% kill
100	New	30–45 min.	58·0	—	—	31	54·1	63·0	44·0	69
100	New	7 days	60·0	63·0	58·0	0	52·2	63·0	40·0	100
50	—	Controls	60·0	63·0	58·0	48	52·2	63·0	40·0	4
50	7 days	3 hr.	58·0	—	—	16	50·6	58·0	40·0	68
50	7 days	24 hr.	57·5	58·0	57·0	9	50·6	58·0	40·0	82
50	7 days	7 days	56·0	58·0	54·0	3	52·0	58·0	40·0	94
50	7 days	Controls	56·0	58·0	54·0	50	50·6	58·0	40·0	0

Table IV does not contain sufficient data to decide the minimum period of exposure which will give a maximum toxic effect for the particular film, but indicates that an exposure of 24 hours to films up to 1 week old will give fair percentage kill. It suggests that new films would be effective in a few hours. Further evidence on these points was obtained in the laboratory experiments.

Finally a short series of experiments was made to test the lasting properties of the film during the summer months, in case these properties should differ very greatly from those of films prepared in the colder weather. The results of these are shown in Table V.

Table V

Experiment to test the lasting properties of films of 0·8% pyrethrins in Shell oil 24210 applied in the summer at the rate of approximately 0·0053 g./sq. in. to boxes of Canadian hemlock. The films were kept in the dark. The insecticide was made up from an extract standardized by the Seil acid method. The test insects used were the migrating larvae of *P. interpunctella*. Experiments carried out between 9 August and 8 September.

No. of larvae	Age of film	Period of exposure to film	Temperature range during exposure (° F.)			No. surviving to pupate	Temperature range during experiment (° F.)			% kill
			Av.	Max.	Min.		Av.	Max.	Min.	
100	12 days	4 days	70·6	73·0	68·0	0	67·0	74·0	62·0	100
50	19 days	7 days	65·3	68·0	62·0	0	66·0	70·0	62·0	100

Table V shows that the film remains fully effective in the summer for periods of at least 19 days. The results of this experiment taken in conjunction with those of the experiments previously described show that the film will remain effective over a considerable period when kept at the normal temperatures of the English climate, in the dark or in diffused light.

Laboratory experiments

(*a*) *Experiments on migrating larvae of* Plodia interpunctella *Hb.*

A short series of experiments was made in the laboratory under more completely controlled conditions than it was possible to achieve in the semi-field experiments described above.

Small squares of Canadian hemlock of either 4 or 4½ in. were cut out and weighed. Each square was then sprayed in a spraying tower, which had been designed for testing contact insecticides. They were then re-weighed, the difference in weight giving the amount of spray deposited. The tower has been tested to make certain that the spray is evenly deposited over the square.

The squares prepared in this way were either put together to form a box in which the test insects were placed, or they were used singly. In the latter case the insects were put on the film and prevented from wandering off by means of a down-turned petri dish or filter funnel.

The standard adopted for the film was 0·0065 g./sq. in. of insecticide which was slightly higher than that in the semi-field experiments, since at least two applications should be made before moth emergence com-

mences. This amount corresponds to one heavy spraying. Owing to the difficulties of experimental technique, the standard could only be approximated. The actual concentrations are given for each experiment and the variations do not seem to be significant.

The first series of experiments were designed to amplify the data obtained in the semi-field trials on the minimum length of exposure of the insects to new films, necessary to obtain a maximum toxic effect. The results are given in Table VI.

Table VI

Experiment to determine the length of exposure of migrating larvae of *P. interpunctella* to new films of 0·8% pyrethrins in Shell oil 24210 on squares of Canadian hemlock necessary to kill. Three squares were used, the larvae being distributed over the three. Concentration of insecticide: Square 1, 0·0064 g./sq. in.; Square 2, 0·0072 g./sq. in.; Square 3, 0·0077 g./sq. in. Temperature during experiment: 68–72° F. Insects subsequently kept in an incubator at 73–75° F. and 70% R.H.

Time larvae exposed to film (min.)	No. used	No. surviving	% kill	Approximate survival period (days)
15	25	8	68	27
30	25	1	96	27
60	25	0	100	27
120	25	0	100	27
240	25	0	100	27
360	25	0	100	27
Controls	25	19	24	27

Table VI shows that an exposure period of 1 hour to a fresh film was completely effective under the conditions of the experiment.

It was obvious from the previous experiments that the activity of the film diminished rapidly with age, and a series of experiments was therefore made to test the minimum effective length of exposure to films 1 week old. The results of this series are given in Table VII.

Table VII

Experiments to determine the length of exposure of migrating larvae of *P. interpunctella* to 7-day-old films of 0·8% pyrethrins in Shell oil 24210 on squares of Canadian hemlock made up into a box, necessary to kill. Concentration of insecticide, 0·0062–0·0077 g./sq. in. Temperature during experiment, 68–72° F. Insects subsequently kept in an incubator at 73–75° F. and 70% R.H.

Time larvae exposed to film	No. used	No. surviving	% kill	Approximate survival period (days)
15 min.	25	21	16	27
30 min.	25	19	24	27
60 min.	25	18	28	27
120 min.	25	17	32	27
240 min.	25	17	32	27
360 min.	25	18	28	27
24 hr.	25	0	100	27
48 hr.	25	0	100	27
72 hr.	25	1	96	27
Control	25	25	0	—

Table VII confirms that the activity of the film has greatly decreased on keeping 7 days. A 6 hr. exposure only gives a very small kill. An exposure period lying between 6–24 hr. is necessary to secure a kill approximating to 100%.

(*b*) *Summary of the semi-field and laboratory experiments on the action of the film on migrating larvae*

A film of 0·005–0·007 g./sq. in. of insecticide on an absorbent wood, Canadian hemlock, will give either a complete or an almost complete kill of the larvae of *P. interpunctella* and, therefore, also of the more susceptible larvae of *E. elutella*.

The film will remain effective for at least a week and retains a high degree of toxicity for at least 19 days at temperatures commonly prevailing in England. The period of exposure of the insects to the film necessary to obtain a maximum effect varies with the age of the film. An exposure of 1 hour to freshly prepared films is enough to secure a high percentage kill, but a period of between 6 and 24 hours is necessary for week-old films. The pyrethrum is the main toxic agent, the oil in the insecticide having little or no effect.

These data apply to films that are in the dark or in diffused daylight.

(*c*) *Laboratory experiments on the moths of* Plodia interpunctella

It has been stated, previously, that practical control is obtained by the action of the film on the moths, the action on the larvae being of secondary importance. When, therefore, the above information on the activity and lasting properties of the film had been obtained by experiments with the larvae, it was necessary to get definite information on the susceptibility of the adults. Once a comparison had been made between the susceptibility of the larval and the adult instars, the results of the experiments made on the larvae could be applied to the adults. A series of experiments was, therefore, carried out in the laboratory to test the susceptibility of the moths of *P. interpunctella* to films of insecticide. Preliminary experiments showed no appreciable difference in the susceptibility of moths of *E. elutella* and *P. interpunctella*, so the latter were used, because they were more readily available.

The experiments were made whenever moths were available, because of the difficulty of obtaining large numbers of known age at any given time. In spite of this lack of standardization of the biological material, the experiments should afford a useful index of the effect of the films on

the moths; it is noteworthy that none of the 375 moths exposed to the films survived.

When moths are placed on new or old films they are rapidly paralysed. The most resistant moths were paralysed in 30 min. on an old film; 15 min. is usually sufficient on fresher films. The moths remain in a state of paralysis until they die, although signs of life consisting only of an ability to twitch the legs and antennae may persist for several days or as long as the normal adult life. Controls were kept during each experiment to ascertain that no deaths would normally occur during the experiment. The treated moths were kept to see whether any would recover, but none did. The method of making the films was similar to that described above for the experiments on the larvae. The results are given in Table VIII.

Table VIII

Results of experiments on the effect of films of 0·8% pyrethrins I and II in white oil applied to wood of Canadian hemlock on moths of *P. interpunctella*. Experiments made in the laboratory at temperatures between 60 and 70° F.

Age of film	Amount of insecticide in g./sq. in.	No. of insects used	Age of insects	Period before insects moribund (min.)	Period insects exposed to film (min.)	% kill
30 min.	0·0062–0·0072	25	Unknown	8	15	100
Controls	—	25	,,	—	—	0
6 hr.	0·0064–0·0077	50	,,	10	10	100
24 hr.	0·0058–0·0070	16	,,	6	6	100
Controls	—	13	,,	—	—	0
8 days	0·0062–0·0072	50	,,	10	10	100
Controls	—	25	,,	—	—	0
8 days	0·0064–0·0075	37	1–3 days	14·5	15	100
Controls	—	15	,,	—	—	0
14 days	0·0058–0·0070	16	Unknown	24	33	100
Controls	—	8	,,	—	—	0
17 days	0·0062–0·0072	33	1–6 days	13·5	15	100
Controls	—	10	,,	—	—	0
19 days	0·0058–0·0070	16	Unknown	—	30	100
Controls	—	4	,,	—	—	0
26 days	0·0058–0·0070	14	,,	14	30	100
Controls	—	8	,,	—	—	0

(*d*) *Summary of the experiments on moths*

These experiments indicate that a film of the insecticide within a range of 0·0058–0·0077 g./sq. in. on an absorbent wood, such as Canadian hemlock, will remain effective against these moths for at least 26 days, if protected from dust and strong sunlight.

A comparison of the results in Table VIII with those of Table I–VII shows that the moths of this species are very much more susceptible than the full grown migrating larvae.

The success of the process in practice is due to the great susceptibility of the moths, against which stage in the life-history the method is directed.

Preliminary experiments on the effect of pyrethrum-white oil films on miscellaneous insects

After it had been found that pyrethrum-white oil films were highly toxic to the adults and larvae of *P. interpunctella* and *E. elutella*, a few preliminary experiments were made with these films on other species of indoor insects.

The experiments were done in the laboratory and the technique was similar to that used for *P. interpunctella*. Small squares of Canadian hemlock were coated with the insecticide and the material was allowed to soak into the wood for a period of not less than half an hour. The insects were placed on the film after the first half hour and before the lapse of 24 hr.

In the first series of experiments the squares were made up into a box and the insects were placed inside it; in the later series single squares were used and the insects were confined to the square by means of an upturned petri dish or filter funnel. The insects were not standardized in any way, but were of varying ages and had been bred under a variety of conditions. The experiments can, therefore, only be regarded as a guide to the relative susceptibility of the various species and the figures for percentage kill as rough approximations. The results of these experiments are given in Tables IX*a* and IX*b*.

Table IX*a*

Preliminary experiments on the effect on miscellaneous insects of films of 0·8% pyrethrins in white oil on Canadian hemlock. Films not more than 24 hr. old. Temperature range during the experiments, 60–66° F.

Species	Instar	Amount of insecticide in g./sq. in.	Time of exposure	No. of insects used	No. surviving	% kill
Lasioderma serricorne F.	Adults	0·0064–0·0076	15 min.	27	2	92·6
	,,	—	Controls	25	20	20
Sitodrepa panicea R.	,,	0·0062–0·0072	30 min.	26	0	100
	,,	—	Controls	25	22	12
Tineola biselliella Hüm	,,	0·0062–0·0072	15 min.	16	0	100
	,,	—	Controls	10	9	10
	Larvae	0·0062–0·0072	30 min.	25	0	100
	,,	—	Controls	25	23	8
Tribolium castaneum Hort.	Adults	0·0064–0·0076	15 min.	23	0	100
	,,	—	Controls	25	24	4
Trogoderma versicolor Creutz	,,	0·0062–0·0072	30 min.	26	1	96·2
	,,	—	Controls	25	22	12
	Larvae	0·0062–0·0072	30 min.	25	24	96
	,,	—	Controls	25	25	0
Oryzaephilus surinamensis L.	Adults	0·0068	30 min.	25	0	100
	,,	—	Controls	25	22	12

Table IX *b*

Preliminary experiments on the effect on *Cimex lectularius* L., *Calandra granaria* L. and *C. oryzae* L. of films of 0·8% pyrethrins in white oil on Canadian hemlock. Films not more than 24 hr. old. Temperature range during the experiments, 60–66° F.

Species	Instar	Amount of insecticide in g./sq. in.	Time of exposure	No. of insects used	No. surviving	% kill
Cimex lectularius L.	Adults	0·0062–0·0072	30–45 min.	24	1	95·8
	,,	—	Controls	25	25	0
	,,	0·0064–0·0076	60 min.	23	5	78·3
	,,	—	Controls	25	25	0
	,,	0·0065	24 hr.	52	0	100
	,,	—	Controls	51	51	0
	Nymphs	0·0065	24 hr.	53	0	100
	,,	—	Controls	50	50	0
	Adults	0·0131	24 hr.	49	0	100
	,,	—	Controls	52	51	1·9
	Nymphs	0·0131	24 hr.	50	0	100
	,,	—	Controls	50	50	0
Calendra granaria L.	Adults	0·0065	2 hr.	21	7	66·7
	,,	—	Controls	24	16	33·3
	,,	0·0067	24 hr.	53	0	100
	,,	—	Controls	50	49	2
	,,	0·0129–0·0132	24 hr.	77	0	100
	,,	—	Controls	71	59	16·9
Calandra oryzae L.	,,	0·0065	24 hr.	52	0	100
	,,	—	Controls	50	49	2

Tables IX*a* and IX*b* show that the adult stage of a number of indoor pests is very susceptible to the action of the film, but *Calandra granaria*, *C. orzyae* and *Cimex lectularius* are important exceptions and the larvae of *Trogoderma versicolor* are also very resistant.

Some additional experiments were carried out on the adults of the two species of *Calandra* and the adults and nymphal stages of *Cimex lectularius* because of the economic importance of these insects. These results indicate that a complete kill of these species may be obtained with a long exposure, but it must be remembered that only small numbers of insects are used so that the results are only indicative. The experiments suggest that the film may be useful against a variety of industrial insects. It might be of particular value in cleaning up the residual infestation in empty warehouses and other buildings where fumigation is difficult or undesirable. However, before concrete recommendations can be made, work must be done on the susceptibility of the various species. Preliminary experiments indicate that a film containing an organic thiocyanate may be more toxic to *Cimex lectularius* than a pyrethrum film, so that by the choice of a suitable insecticidal agent, the method might be useful against all species of indoor pests.

Field scale experiments on E. elutella and P. interpunctella

The results of the preliminary experiments showed that if the boxes and exposed surfaces in a warehouse were sprayed directly by means of atomizing guns, a film of insecticide could be formed which would be lethal to insects settling upon it and would act as a protection to the goods inside the boxes against infestation from without. The film would also prevent infestation spreading outwards from inside the box.

One warehouse in Manchester of a capacity of 268,000 cu. ft. was available for the experiment. It stored approximately 650 tons of dried fruit annually. This warehouse was infested with *E. elutella* and *P. interpunctella*, chiefly the former. The object of the experiment was to prevent the moths emerging from hibernating larvae in the premises from reaching and infesting the stored dried fruits. The fruit had all been fumigated on arrival and was substantially if not completely free from infestation.

The method adopted was to start applying the film about the middle of May, approximately a fortnight before the first moths would emerge and to apply it twice a week during the emergence period of the moths.

The apparatus which was used is of the type already described (Potter, 1935) for use in the mist method. For the present method a small air compressor is sufficient, because it is not necessary to put a large quantity of material into the warehouse in a short space of time, as in the mist method. The air compressor was driven by a one h.p. motor and delivered about 6 cu. ft./min. of free air. This machine cost about £40 and works one gun. In this instance an H.M. oil-spray gun of Aerograph Co. was employed. The air pressure was about 45 lb./sq. in.

No definite rule can be laid down for adjusting the degree of atomization, because it will depend on the accessibility of the area to be coated; in general, as coarse an atomization as possible consistent with even coating and lack of staining is desirable.

(*a*) *Experimental procedure*

Spraying was carried out twice a week from 15 May to 30 September 1936. From 15 May to 16 June a strength of about 0·65% pyrethrins in white oil was used, but from then onwards the strength was raised to 0·8% pyrethrins. An average quantity of 9200 c.c. of spray was used for each spraying of the warehouse of 268,000 cu. ft. and its contents; the contents varied in amount.

(*b*) *Assessment of results*

The results may be assessed in two ways. First, by inspection of the fruit for infestation, together with an inspection of the premises for the bodies of the moths which would have attacked the fruit; secondly, by a comparison of the infestation occurring in the year in which the process was used, with that of the previous year when no control measures had been adopted in the warehouse. Both these assessments were made.

The bodies of the moths which had been killed by the film, when they could be found, were collected and examined to determine the identity of the moths and their sex. The results of this work are given in Table X.

Table X

Results of examination of the bodies of moths collected from the Manchester warehouse during the 1936 season.

Ephestia elutella		*Plodia interpunctella*		*Borkhausenia pseudospretella* St.		*Ephestia cautella* Wlk.		Unidentified	Total	Total wings not expanded
♂	♀	♂	♀	♂	♀	♂	♀			
96	88	2	4	1	2	0	1	28	222	130

The numbers recorded in the above table are not large, but they represent only a small proportion of the moths which were present, since the majority were in inaccessible places and could not be examined. Experience has shown that, if numbers such as these can be found, there are sufficient moths actually present to set up a very serious infestation.

(*c*) *Summary of results of the field scale experiments*

An interesting point brought out by Table X is the high proportion of moths which were killed before they were able to expand their wings. The following explanation may account for this: as soon as the moths emerge from the pupae they crawl on to an exposed surface to expand their wings; this brings them into contact with the insecticidal film and a considerable proportion of them are disabled and killed before they have expanded their wings. This fact also suggests very strongly that the film formation is the effective part of the process and that the moths have not been killed by direct action of the spray.

Three careful inspections were made of the stored fruit in 1936, on 8 June, on 15 July and on 3 September. Another inspection was made on 14 March 1937. These were in addition to those made by the warehousemen during the normal operations of moving the piles and sampling the cases of fruit. Throughout the season no infestation was found.

As evidence of the difference between the state of the fruit when film spraying had been adopted and when no protective measures had been taken, as in 1935, it can be quoted that inspections in 1935 showed that every pile of fruit was infested to a greater or lesser extent. It was necessary to refumigate 56·375 tons during the 1935 season out of a total of 661·885 tons. This suggests that the film spraying was effective, since there was as much chance of infestation in 1936, when none occurred, as in 1935.

General summary and conclusions

1. A series of experiments and a field scale trial have been made of a film spraying method for the protection of stored goods from infestation by *E. elutella* and *P. interpunctella*.

2. It is shown that where the goods have been efficiently sterilized before storing, reinfestation by these two species in the warehouse can be prevented completely, as far as could be ascertained.

The procedure is to spray the interior of the warehouse and the cases stored in it with an insecticide consisting of a solution of 0·8% pyrethrins in a white oil, the constitution of which is given. The apparatus and method of spraying are described. Spraying should be started some time before the moths are expected to emerge; in England about 15 May, and should be continued twice a week throughout the period of moth emergence, which may continue until 30 September.

3. The laboratory and semi-field scale experiments, together with data obtained during the commercial working of the process, show that control is obtained by a protective film of insecticide, which is formed and maintained throughout the period of moth emergence.

A film of about 0·006–0·007 g. of insecticide per sq. in., which is about the amount applied in practice in a single heavy spraying or two light sprayings, on an absorbent wood such as Canadian hemlock, will remain effective against moths of the two species for at least 26 days. It is highly effective against the migrating larvae for a week or longer.

The moths were paralysed and ultimately killed by an exposure to the insecticide of not more than 30 min.; the larvae need to be exposed to the action of the film for periods of 1–24 hr., depending on the age of the film.

4. It was shown that the oil plays very little part in the toxic effect of the film.

5. A short series of experiments indicate that the method of using a protective film of insecticide might be usefully applied in the control of

other indoor insects. Probably its most useful application is in cleaning up the residual infestation in empty warehouses and other buildings, in cases where fumigation is not practicable or desirable.

6. The effectiveness of this method of prevention of infestation by *E. elutella* and *P. interpunctella* has been proved by its adoption for the protection of 30,000 tons of dried fruits in warehouses in London, Liverpool, Manchester and Glasgow, with complete success.

7. The method has also been adopted for the protection of cacao and tobacco in London and elsewhere. In these instances, although a great reduction of infestation was observed, it was not possible to assess the results accurately, because the goods were not sterilized before being brought into store and therefore fresh infestation was continuously brought into the warehouse.

8. The advantages of the method are that it can be used without interference with the normal routine of storage and involves no extra handling of the goods.

It is also a continuous protection from infestation during periods when this is likely to occur. This is a very important point when goods are stored in places where other infested goods may also be stored.

This method has the further advantage, that it will localize and check the spread of infestation if this is brought into a building in which the method is used.

Finally, compared with fumigation, it is cheap and is devoid of risk to human beings.

9. Although the insecticide described in this paper has been successful, it is not necessarily the only possible one for use in this method. Preliminary trials have shown that one of the organic thiocyanates, β butoxy β' thiocyanodiethylether has a marked toxicity when applied in this way and may compare favourably with pyrethrum for some purposes.

I am greatly indebted to Mr J. J. Scouler, Secretary of the Australian Dried Fruits Board, for giving me every facility in the warehouses storing the Board's dried fruits, and to Messrs A. E. Green and C. R. Rainbird, warehouse representatives of the Board, for their assistance. My thanks are also due to Mr Tomlinson, Manager of the Manchester warehouse, for his willing cooperation in the conduct of the field experiment.

The standardization of the pyrethrum extract was carried out in Dr Tattersfield's laboratory at the Rothamsted Experimental Station, and I am indebted to Dr Tattersfield for his courtesy and to Dr Martin for his help in the work of the estimation.

The work was carried out from the Entomology department of the Royal College of Science, under the direction of Prof. J. W. Munro.

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INVESTIGATIONS UPON THE CONTROL OF OAT SICKNESS BY THE ADDITION OF CERTAIN CHEMICAL SUBSTANCES TO SOIL INFECTED WITH *HETERODERA SCHACHTII* SCHMIDT

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(With Plate XXXVI)

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1. Introduction

In 1935 a paper on the nematode *Heterodera schachtii* with special reference to the oat race in Britain was published in the *Journal of Helminthology* (Edwards, 1935). The general field observations made of infested areas in Shropshire during 1933 and 1934 were discussed, as well as the results of experiments designed to ascertain the range of host plants liable to attack by this strain. It was shown that great practical difficulties would be involved in planning suitable crop rotations whereby the oat race could be controlled, since the parasite is capable of attacking and reproducing freely not only on cereals but also on rye-grasses and red clover. In view of these circumstances, the problem of affording direct protection to cereal crops against invasion of the nematode by the addition of chemical substances to affected soil was next investigated in 1936 and 1937. The present communication records the results obtained during this subsequent study.

2. Method

The method adopted to determine the protective value of the different materials was to grow oats in earthenware flower pots, 11 in. in diameter, which were filled to within 1 in. of the top with soil previously treated with the respective dressings. The soil used, the same in all pots, was heavily infested with the cereal race of *H. schachtii*, and had been taken from a field that was worthless for the growing of cereals on account of "oat-sickness". The field was known to have produced until recent years excellent yields of grain and was still in a satisfactory state of fertility, judging by the growth of other crops and by the chemical analysis of the soil. The latter was as follows:

	%
Organic matter	4·11
Total nitrogen	0·20
Available phosphoric acid (P_2O_5)	0·021
Exchangeable potash (K_2O) ...	0·016
Carbonates (as $CaCO_3$)	0·210
Lime requirement ($CaCO_3$) ...	Nil
*p*H	7·1

These data show that there was no deficiency in essential "plant food", which might account for the failure of the cereal crops.

When the soil was air-dried it was lightly pulverized and all the coarser material removed with a $\frac{1}{3}$ in. sieve. The soil was then thoroughly mixed until it became uniform in general appearance and cyst concentration. When the cyst counts of several consecutive samples of identical weight taken at random from the heap of soil were compatible with one another, it was assumed that homogeneity with regard to cyst distribution had been attained.

Special precautions were taken to obtain a thorough incorporation of each experimental substance with the entire soil of its respective pot. The contents of each pot selected for treatment were turned out on to a clean sheet of strong brown paper on a table and the chemicals incorporated with the soil by hand. The resulting small heap was then passed five times through a sieve of $\frac{1}{3}$ in. mesh, care being exercised that no two consecutive trowelfuls came from the same part of the heap. All signs of "stratification" due to unevenness of distribution had disappeared after the third sifting. After this intimate admixture with the chemical, the soil was returned to the pot and kept moderately moist until the sowing of the oat seed.

The tests were carried out in triplicate, a series of three pots being allotted to each treatment, together with a similar series left untreated. An application of artificial manure at the rate of 6 cwt. to the acre was given to all the pots, including the controls, a few days before sowing. It was composed of two parts of sulphate of ammonia, three parts of superphosphate and two parts of sulphate of potash. All the pots were sown, with the variety "Victory" in the first week in April, at the rate of 30 seeds per pot at a depth of $\frac{1}{2}$ in. They were then plunged in groups, according to their series, in a bed of ashes and soil in an open air enclosure with a south-westerly aspect, being carefully watered throughout the season in such a way as to approach as closely as possible field conditions.

At intervals during the course of the experiments, quantitative observations were made on the plants in all series to obtain an analysis of the effects of different treatments upon (*a*) germination, (*b*) vegetative growth, (*c*) panicle production and yield of

grain, and (*d*) degree of infestation of the roots by the nematode. Of these four factors vegetative growth (*b*) proved the least satisfactory, since it was not found possible to assign to it any definite standard of measurement apart from that given by the number of plants and average height of shoots at two distinct periods during their early development.

3. Treatments tested

The following substances were intimately mixed with the soil of the respective series of pots:

1. Ferrous sulphate at the rate of 15 cwt. per acre.
2. Precipitated ferric oxide at the rate of 6 cwt. per acre.
3. Natural ferric oxide at the rate of 6 cwt. per acre.
4. Ferric chloride at the rate of 12½ cwt. per acre.
5. Nitrate of soda at the rate of 60 cwt. per acre.

6–10. Calcium cyanamide at rates of 20, 40, 60, 80 and 100 cwt. per acre, respectively.

The ferrous sulphate, ferric chloride and the different dressings of calcium cyanamide were added to the soil 25 days before the seed was sown, and the ferric oxides as well as the nitrate of soda at the time of sowing.

The various compounds of iron were included in the investigation in view of the promising results given by them in experiments conducted by Hurst & Triffitt (1935) on control of the potato sickness associated with *H. schachtii*. The nitrate of soda series was prepared with the object not only of observing whether this nitrogenous fertilizer would in any way assist the plants to overcome the adverse effects of oat sickness, but also for purposes of comparison with the calcium cyanamide series. Since the addition of the calcium cyanamide would considerably increase the nitrogen content of the soil, any beneficial results obtained by its use might be due to the abundance of available nitrogen.

4. Discussion of data obtained in 1936

(1) *Effect of treatments on vegetative growth*

It was possible throughout the growing season to detect marked differences in the growth and vigour of the oat plants under the various treatments. The plants in the untreated soil produced normal growth for the first few weeks, but this was followed by a disastrous check in the growth rate accompanied by the customary manifestations of the oat-sickness associated with root invasion by *Heterodera schachtii*.

For some weeks after germination, the plants in the series of pots

treated with calcium cyanamide in amounts exceeding 40 cwt. to the acre showed definite indications of distress, being fewer in number (Table I series 4, 5, 6), somewhat irregular in size, and exhibiting a definitely slower rate of growth than the controls. These adverse effects were in direct relation to the amount of the chemical added to the soil, being most pronounced in the pots which had been given the heaviest application. This is well exemplified by the average height of the shoots recorded for the plants under the different treatments some seven weeks after sowing as shown in the sixth column of Table I. It will be seen that the shoot growth in the series (3, 4, 5, 6) which had received dressings of cyanamide corresponding to 40, 60, 80 and 100 cwt. per acre measured on an average 12, 25, 45 and 56 mm. less in height respectively, than that of the plants in the untreated soil. From this time onwards, growth in the pots treated with these heavier dressings of cyanamide gradually accelerated, until by mid-July the plants possessed foliage conspicuously superior to that of the controls, both in regard to its amount and the depth of colour. The average height of the shoots in each series at this later date is given in the seventh column of Table I where it will be noted that the shoot growth for each of the four treatments averaged 190 mm. between 16 June and 10 July, as against 52 mm. for the controls during the same period, that is, the growth rate in height of the plants had been more than trebled by these soil dressings. This pronounced disparity was maintained until the plants had attained full maturity and were finally examined.

On the other hand, at the outset the plants in the ferrous sulphate (series 9), and particularly in the ferric chloride (series 10) pots developed more rapidly than those in any of the other treated ones or in the controls (series 1). As the season progressed, this stimulating influence on the rate of foliage growth became more and more apparent and by mid-June was most striking. It was calculated that these plants were at this date approximately 7 days more advanced in growth than those in the control pots; there was on 16 June a difference on an average of 48 and 75 mm. between the height of the plants in the controls and those in the ferrous sulphate and ferric chloride soils, respectively (see Table I, sixth column). After the second week in June, this difference gradually became less apparent and by mid-July had become reduced in both instances to about 25 mm. (Table I, seventh column). There could be no doubt from the general appearance of the foliage that the plants in both these treated series were, at this stage, seriously affected by oat sickness although marked symptoms had been absent heretofore.

Table I. *Effect of different treatments on (a) establishment, growth and yield of oats in* 1936, *and (b) infestation of the roots of oats by* H. schachtii *in* 1936 *and* 1937

Series	Treatment	Serial no. of pot	No. of plants established		Average height of shoots		No. of panicles	No. of fertile grain	Wt. of fertile grain	No. of cysts on outer roots	
			12 May	15 Sept.	16 June cm.	10 July cm.			g.	1936	1937
1	Untreated (control)	1*a*	26	13	12·5	17·5	7	19	0·52	35	142
		1*b*	24	10	12·6	17·7	7	21	0·56	38	153
		1*c*	25	12	12·8	18·1	8	23	0·63	43	149
2	Cyanamide 20 cwt.	2*a*	27	24	16·6	34·6	23	184	5·73	89	151
		2*b*	26	23	16·3	34·2	23	174	5·24	79	141
		2*c*	24	22	16·6	34·3	22	170	5·42	85	146
3	Cyanamide 40 cwt.	3*a*	21	20	11·2	30·0	20	252	8·39	22	91
		3*b*	24	22	11·4	30·4	19	233	7·74	28	105
		3*c*	23	23	11·6	30·5	22	241	8·06	26	102
4	Cyanamide 60 cwt.	4*a*	21	21	10·4	29·2	21	285	9·34	13	78
		4*b*	20	20	10·0	28·6	18	272	8·90	12	78
		4*c*	20	19	9·9	28·8	19	269	8·84	9	69
5	Cyanamide 80 cwt.	5*a*	17	17	8·0	27·4	17	285	9·39	0	48
		5*b*	19	18	8·5	27·4	18	283	9·45	0	49
		5*c*	18	17	7·8	26·9	16	271	8·95	0	44
6	Cyanamide 100 cwt.	6*a*	18	17	6·6	25·9	17	278	9·07	0	0
		6*b*	19	19	7·4	26·5	19	296	9·69	0	0
		6*c*	18	18	7·1	26·2	17	284	9·25	0	0
7	Precipitated ferric oxide	7*a*	24	14	13·7	17·9	8	47	1·23	52	141
		7*b*	25	16	13·8	18·5	11	45	1·24	56	149
		7*c*	26	14	14·1	18·1	7	38	1·20	46	150
8	Natural ferric oxide	8*a*	25	14	13·7	18·3	10	36	0·92	48	151
		8*b*	24	11	13·2	17·7	7	32	0·93	47	148
		8*c*	22	12	13·5	17·7	8	29	0·89	43	140
9	Ferrous sulphate	9*a*	21	12	17·5	20·3	9	59	1·79	55	147
		9*b*	23	16	17·6	20·5	12	71	1·98	64	152
		9*c*	24	14	17·2	20·0	10	57	1·86	56	143
10	Ferric chloride	10*a*	23	17	20·3	20·5	13	76	1·99	67	149
		10*b*	22	15	20·2	20·4	10	67	2·08	61	131
		10*c*	25	17	19·9	20·3	12	72	1·93	60	144
11	Sodium nitrate	11*a*	22	16	13·6	18·1	10	34	0·89	46	139
		11*b*	24	15	13·7	18·7	13	30	0·90	41	147
		11*c*	25	18	14·0	18·3	11	29	0·88	42	153

The plants in the series treated with nitrate of soda and with precipitated and natural ferric oxides (nos. 7, 8, 11), were essentially similar to one another in general appearance throughout the season, and showed but little improvement over the controls. In contrast, those growing in soil which had received calcium cyanamide at a rate equal to 20 cwt. per acre were, in comparison with the controls, at all times characterized by their excellent growth rate and, as in the case of the other cyanamide treated series, by the dark green colour of their foliage as well as an entire absence of symptoms suggesting the presence of oat sickness.

(2) *Effect of treatments on yield of crop*

The produce was harvested from each pot on 15 September, and the numbers of plants and panicles recorded. When the oat grains were being threshed out some weeks later, the total yield from each pot was weighed out separately. The tabulated results are set out in Table I, where it will be seen that the degree of oat sickness was of a severe and fairly uniform order throughout the soil used in the present experiments, judging by the yields of grain obtained in the different control pots. From the 30 seeds sown in each of the three untreated pots the number of plants produced out of the possible numbers were, respectively, by 12 May, 87, 80 and 83 % and at harvest time 43, 33 and 40 %. Between these dates the control pots lost on an average 44 % of their plants, no doubt mainly due to the effects of oat-sickness. Further serious features associated with the disease are demonstrated in the control pots by the poor panicle production per unit area, and by the low weight of grain produced per panicle. Of the plants at harvest time in this untreated series, approximately a third failed to produce panicles, even though they were fewer in numbers at that date per unit area, and consequently, had more space for better shoot development than those in the cyanamide series.

The figures in the final columns of Table I indicate that all the soil treatments tested had some effect upon the crop, though in some instances, the differences are probably not significant. The greatest increase in weight of grain per unit area was found in those pots which received calcium cyanamide in dressings exceeding 40 cwt. to the acre, being on an average for these three series (4, 5, 6) 9·21 g. per pot compared with 0·57 g. per pot for the untreated soil. The same series also gave the highest number of fertile grains per panicle, averaging 15·8 as against 2·9 per ear for the controls, and 9·7 in the two series (2, 3) treated with cyanamide at the lower rates. It is difficult, however, to say how far the larger numbers, and therefore, more crowded condition of the

plants in the less heavily dressed series (2, 3) might have influenced the final results. The average numbers of plants per pot at harvest time were 18·4 for series 4, 5 and 6, and 22·3 for series 2 and 3. The significant differences in the yields of crop in the various cyanamide-treated series might therefore be due, at least to a certain extent, to the factor of competition, and not entirely to the degree of protection afforded to the plants from the deleterious effects of oat-sickness.

The data in Table I, concerning the relative value of the substances applied, other than calcium cyanamide, indicate that the effects upon panicle production and yield of grain, at the concentration tested, are doubtfully significant. As in the control series, seed of small size and poor quality was produced. Calcium cyanamide, on the other hand, favoured the development of the ear so that not only did it produce crops with high numbers of grains per panicle, but also produced the largest grains under the conditions of the experiments.

It would seem that the complete failure of the various dressings, other than calcium cyanamide, to afford appreciable protection of the plants from the pathological conditions associated with oat sickness, in the circumstances described when each was intimately mixed with the soil and given the desired degree of moisture, holds out little hope of ultimate success in combating the disease by means of any of these treatments when working on a field scale.

(3) *Effect of treatments on infestation by the nematode*

The technique adopted in the present investigation to determine the effects of the various soil treatments on the degree of infestation of the plants by the nematode was to count the number of encysted females actually protruding from the exterior of the roots (Triffitt, 1929). For the purpose, the contents of each pot were carefully turned out in an intact mass on 5 August and examined. At this date the major proportion of the root system was exposed upon the surface of the mould of soil and the white-stage females were clearly visible. The cyst counts carried out in this manner on the oat plants are set out in Table I, column 11, where it will be noted that none of the substances tested, with the exception of calcium cyanamide, had reduced the amount of eelworm infestation as indicated by the number of cysts on the roots. The most successful results are seen to have followed the application of calcium cyanamide in dressings at the rate of not less than 80 cwt. to the acre when production of cysts was completely suppressed (series 5, 6). A high degree of efficiency, as indicated by freedom from cysts on the roots, was

also obtained by the use of cyanamide at 60 cwt. per acre (series 4), the cyst population being only 28·2 % of that of the plants in the untreated series. Pl. XXXVI, Fig. 1 shows the marked difference between the root systems of typical plants from series 4 and from the control series. The inhibitory influence of cyanamide on cyst formation was decidedly less marked where it was used at the rate of 40 cwt. per acre, the infestation having only been decreased by about a third (series 3).

In contrast to the heavier dressings of calcium cyanamide tested, an application corresponding to 20 cwt. to the acre actually increased the nematode infection, the number of cysts on the roots being rather more than double the average control count. Slight increases in the eelworm infestation of the roots also followed all the other treatments included in the experiments. The intensity of the infection was, in general, directly in proportion to the vigour of the host plants. Thus, the series which showed the most luxuriant growth and the highest yield of grain also produced not only the best root development, but supported the largest eelworm population. The experiments were not sufficiently comprehensive to indicate why the oats grown, for instance, in the soil treated with calcium cyanamide at the rate of 20 cwt. to the acre were able to support with relatively little harm a cyst concentration twice as great as that which proved fatal to the plants in the untreated soil. Essentially similar trials carried out by the writer (1937) in 1936 on a field scale, against the potato race of the nematode gave results of the same kind. It was then discovered that the addition of these chemicals to the soil had delayed successfully eelworm invasion during the early growth of the plants, as shown by the time required for the protrusion of the female cysts from the roots, and that a marked correlation existed between the intensity of the sickness and the degree of the inhibitory action produced in this direction by the different treatments. It would seem that, as with potatoes (Carroll & McMahon, 1935), where the onset of the nematode attack is retarded until a good root system has been established, the effect on further growth and yield of the plants is comparatively small and under such circumstances the plants exhibit a high degree of tolerance of *H. schachtii*.

5. Discussion of data obtained in 1937

After harvesting in 1936, the plants in all pots were cut off at ground level, and the roots left undisturbed in the soil until a few days before re-sowing with oats in 1937. The contents of each pot were then turned out, broken up (neither roots nor cysts being removed) and thoroughly

mixed before returning to the pot. Oats were again sown, no treatment other than a dressing of mixed organic fertilizer at the rate of 6 cwt. to the acre being given. The latter consisted of 2 parts sulphate of ammonia, 4 parts superphosphate and 1½ parts sulphate of potash. The same variety ("Victory") was used and the sowing date, 15 April, was the same as in the previous year.

(1) *Effect of treatments on growth and yield*

Growth started quite normally in all pots, irrespective of the chemical treatment given to the soil in 1936. By the end of June considerable difference could be seen, however, between the plants in the series treated with calcium cyanamide in amounts exceeding 40 cwt. to the acre and those in the other series. The former appeared normal, no marked distinction being observed between those in soil treated at the rate of 60 cwt. per acre and those in which heavier dressings of the substance had been used. The other series, including the controls, were closely similar to one another, the plants exhibiting the usual symptoms of heavy parasitization by *H. schachtii*. An exception must be noted in the case of the pots which received calcium cyanamide at the rate of 40 cwt. to the acre. The plants in these pots, though somewhat stunted in growth, did not show symptoms of the disease so noticeably as the controls.

Inspections later in the season confirmed earlier impressions. The plants in the series receiving not less than 60 cwt. of calcium cyanamide per acre remained free from any signs of attack and grew normally with the production of good panicles. The plants of all the other treated series, on the other hand, remained typically diseased and showed at no time any improvement over the controls, except perhaps those grown in soil treated with calcium cyanamide in an amount equivalent to 40 cwt. per acre, but even the best of these could not be considered normal. Unfortunately, the ripening grain was attacked by birds and it was not found possible to obtain reliable numerical data beyond the panicle emergence stage.

(2) *Effect of treatments on infestation by the nematode*

The same technique as in 1936 was adopted to ascertain the influence of the treatments on infestation of the plants by the nematode. The contents of each pot were turned out on 3 August; the detailed figures of cyst counts for all the treatments being presented in the final column of Table I. It will be seen that only the plants grown in soil treated with calcium cyanamide at the rate of 100 cwt. to the acre remained free of

eelworm infection, as indicated by the absence of white stage females on the roots. Unlike the results obtained in 1936, the plants in the series dressed with 80 cwt. per acre of cyanamide bore definite evidence of attack but, on the average, the number of cysts present was only about a third of the count on the controls. Although the application at the rate of 60 cwt. per acre had limited the nematode infestation in 1936 to a negligible amount, and had enabled the production of a vigorous, healthy crop for two seasons after treatment, nevertheless it failed to prevent the eelworm population from increasing by the end of this period to a total nearly twice its original figure, i.e. that found on the control plants in 1936. This dressing had, however, produced appreciable relief even to the plants grown in the second year after treatment, from invasion of the root tissue by the nematode, as indicated by a comparison of the cyst counts recorded for this series in 1937 with those for the control in the same year. It would seem that the beneficial influence which also followed the use of 40 cwt. of calcium cyanamide to the acre, was apparent in the second year also but to a much less extent than in the case of the heavier applications of this substance. None of the other treatments applied in 1936 had been of any value in preventing heavy parasitization of the oats grown in 1937 since, in no instance, did the number of cysts on the roots differ materially from the average control count.

It will be seen from Table I that, as a result of growing oats for two years in succession, a great increase in the number of cysts in the roots exposed on the surface of the mould of soil in the pots occurred, except where the heavier dressings of calcium cyanamide had been administered. In the control series the infection approximately quadrupled; the cyst population in the pots which received 20 cwt. of calcium cyanamide per acre had doubled, that in the sodium nitrate and ferric oxides series had trebled, and that in both ferrous sulphate and ferric chloride series had increased about two and a half times. It must not be inferred from this data, however, that a correlation existed between these dressings (series 2, 7, 8, 9, 10, 11) and the degree of infestation of the oats in 1937, and that, of the treatments given, calcium cyanamide (series 2) was the most successful in protecting the plants from invasion of their root tissues by the nematode since, in this instance, the cyst population had only doubled. A close analysis of the cyst census for the two seasons (Table I, columns 11 and 12), and consideration of the effect of the treatments on the growth made by the plants in 1937 (p. 863), seemed to provide definite evidence that the variations in the rate of increase in the cyst infestation in 1937 were not bound up with any protection afforded

by the materials added to the soil in 1936 but rather, on the one hand, with the original degree of infection of the soil in the respective series (nos. 2, 7, 8, 9, 10 and 11) as indicated by the cyst counts on the roots in 1936 (Table I, column 11) and, on the other hand, by the amount of growth made by the oat plants of 1937. In all instances the rate of increase in the numbers of cysts in 1937 was inversely proportional to the eelworm infestation of the 1936 oat crop. Thus, of all the treatments, the calcium cyanamide series (no. 2) showed the heaviest root infection in 1936 and the least change in the cyst concentration in 1937. The limitation in the degree of increase in the nematode population imposed in each case was, almost certainly, determined by the extent of the growth made by the plants. That the intensity of eelworm infection may be greatly influenced by the vigour or state of the crop seemed to be well exemplified in the present investigations, both in 1936 (p. 862) and in 1937. In the latter year, in all series (nos, 1, 2, 7, 8, 9, 10, 11) except where oats were grown in soil treated with calcium cyanamide at rates exceeding 20 cwt. per acre (series 3, 4, 5, 6), the growth made by the plants in 1937 was closely similar both in regard to its amount and the symptoms suggesting the presence of "oat sickness" (p. 863). These series also exhibited in the same year remarkable uniformity in the degree of attack by the parasite, the average number of encystic females on the outer roots being in each case approximately 148 per pot (Table I, final column).

6. Summary

An account is given of pot experiments carried out in triplicate in 1936 and 1937 upon the control of the oat sickness associated with the Root eelworm, *H. schachtii*. Calcium cyanamide, sodium nitrate, ferrous sulphate, ferric chloride and two forms of ferric oxide were incorporated intimately with infected soil, and quantitative observations were made on the effects of the treatments upon oats grown in the soil in 1936 and 1937 with regard to (*a*) germination, (*b*) vegetative growth, (*c*) panicle production and yield of grain, and (*d*) degree of infestation of the roots by the nematode. The data obtained show that while all the dressings produced better growth of the plants, at least in the first year after application, calcium cyanamide alone yielded significant results. Further, of the treatments tested, only the use of calcium cyanamide at the rate of as much as 100 cwt. to the acre protected the plants completely from oat sickness in both years and, at the same time, apparently eradicated the parasite from infected soil. Unfortunately, such heavy dressings are not a practicable proposition except, perhaps, under special circumstances.

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EXPLANATION OF PLATE XXXVI

Fig. 1. (*a*) Root system of oat plant from pot receiving calcium cyanamide at the rate of 60 cwt. per acre (series 4, 1936). (*b*) Root system of oat plant from control series, 1936. Note abnormal thickening of roots and excessive development of lateral rootlets.

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EDWARDS—Investigations upon the Control of Oat Sickness by the Addition of Certain Chemical Substances to Soil Infected with *Heterodera schachtii* Schmidt (pp. 855–866)

NOTE

A NOTE ON CERTAIN VIRUSES OF THE *CUCUMBER VIRUS* 1 TYPE ISOLATED FROM MONOCOTYLEDONOUS PLANTS

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(With Plate XXXVII)

This note records the isolation of viruses of the *cucumber virus* 1 (J. Johnson) type from lily, hyacinth and tulip, and indicates certain differences between the viruses obtained from these monocotyledonous plants and the type virus as determined by the reactions of young tobacco (*Nicotiana tabacum* var. White Burley) and cucumber (*Cucumis sativus* var. Butcher's Disease Resister) plants.

Lily

In April 1937 plants of "*Lilium formosum*" (*L. longiflorum* var. *insulare*) showing typical mosaic mottling and necrotic flecking of the leaves and severe malformation of the flowers were examined and, following the suggestion of Price (1937*a*) that the virus of lily mosaic was related to *cucumber virus* 1, tobacco and cucumber plants were inoculated. A virus of the cucumber mosaic type was detected, and the findings of Price (1937*b*) received general confirmation. Subsequently the same virus was obtained from samples of mosaic diseased "*L. formosum*", from other localities, and "*L. harrisi*" (*L. longiflorum* var. *eximium*).

The lily virus causes circular, pale green or yellowish local lesions (sometimes delimited by fine necrotic lines but never wholly necrotic) on tobacco leaves 4–7 days after inoculation. At first the lesions are often inconspicuous but, as the leaf ages, they become surrounded by dark green bands and more prominent (Pl. XXXVII, fig. 1). Frequently, the virus remains localized in the inoculated leaves, but occasionally systemic infection results, rarely in a first transfer from lily, but more commonly after several transfers through tobacco, when either localized areas of the upper leaves become infected or a more general mild mottle results.

In cucumber faint circular, yellow local lesions, sometimes necrotic on the cotyledons, have been the only symptom, except in one instance when systemic infection occurred. Transfers from systemically infected tobacco gave strains which regularly became systemic in tobacco, but caused a local reaction only on cucumber. The relationship of the lily virus to *cucumber virus* 1 was proved by the double inoculation of *Zinnia elegans* as described by Price.

Hyacinth

A very similar virus to that obtained from lily was isolated from a single hyacinth plant of unknown variety, from Hampshire, which showed a bright yellow streaking of the leaves. The hyacinth virus gives local lesions on tobacco of a similar type to those caused by the lily virus, but rather more severe and usually surrounded by

necrotic bands, 1 mm. or more in width. Systemic infection has occurred on two occasions, as localized infection of upper leaves and once in a more general form. On cucumber, yellow local lesions, but more severe than those caused by the lily virus, have been the only reaction.

Tulip

A third strain of cucumber mosaic virus has been isolated from tulips, variety Zimmerman's Triumph, obtained from two localities in Middlesex by Mr W. Buddin to whom the writer is indebted for the hyacinth plant and other material mentioned in this note). These showed pale yellow or silver-coloured streaks on the leaves (which were malformed), a stunting of the plant and aborted flowers (Pl. XXXVII, fig. 2).

The tulip strain is characterized by the local necrotic reactions of the test plants. In tobacco, 48 hours after inoculation, local lesions appear as pale, green, sunken spots, up to 2 mm. in diameter, which in a few hours turn brown and later become surrounded by a pale green ring (Pl. XXXVII, fig. 3 (*a*)). In cucumber, local lesions are first apparent after 2–3 days as circular dark green spots with a minute necrotic centre but, later, the spots become necrotic and adjacent lesions coalesce (Pl. XXXVII, fig. 4). No systemic infection of any kind has been observed and the virus has, during a period of 3 months, been subjected to ten successive transfers through tobacco without any change in its reaction. The relation of the tulip strain to the type virus has been demonstrated by the suppression of local lesions on tobacco leaves invaded by the latter virus. (In Pl. XXXVII, fig. 3 (*b*), five local lesions caused by the tulip virus are visible in areas not yet invaded by the type virus. In the series from which the photographed leaf was taken 620 lesions developed on the untreated half-leaves, and 40 on the previously inoculated halves.)

Discussion

The three strains of cucumber mosaic virus noted above show a common tendency to remain localized in tobacco and cucumber and, in this respect, differ from the type virus and most of its variants which become systemic in these hosts. The tulip strain, however, shows a similarity to *Strain* 6 described by Price (1934). The intensity of the necrotic action, which appears to be correlated with the tendency to remain localized, is greatest in the tulip and least in the lily virus, and the three strains could legitimately be classified into two groups, the lily and hyacinth strains being more similar to each other than they are to the tulip strain.

Particular interest is attached to the tulip strain on account of McWhorter's (1937) results showing that one of the viruses responsible for tulip break is related to those infecting lilies. Inoculations have been made to tobacco from the flowers, leaves and bulbs of five samples of tulips (Wm. Pitt, Wm. Copland and Clara Butt) showing various degrees of breaking, but no definite evidence has been obtained of the presence of a virus similar to the one detected so readily in Zimmerman's Triumph.

Inoculation experiments are necessary to prove whether the viruses isolated will cause the diseases in the hosts from which they were obtained, and to elucidate their relationships by cross inoculations. It is hoped that such experiments will be carried out when suitable virus free stocks of plants have been assembled.

No evidence has been obtained that viruses of the cucumber mosaic type are implicated in bulbous *Iris* mosaic, *Narcissus* stripe, *Hippeastrum* mosaic and *Gladiolus* mosaic (probably the disease observed by Dosdall (1928)), while the strains of *cu-*

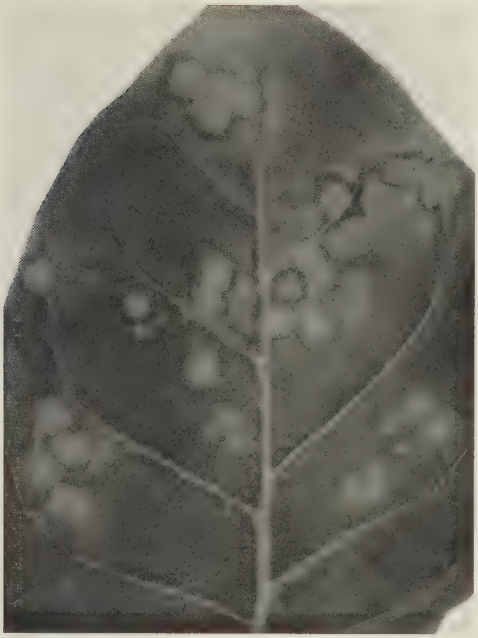

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

AINSWORTH.—A Note on Certain Viruses of the *Cucumber virus* 1 Type Isolated from Monocotyledonous Plants (pp. 867–869)

cumber virus 1 isolated by the writer during the last few years from dicotyledonous plants belonging to several different families have all approximated to the type virus.

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EXPLANATION OF PLATE XXXVII

Fig. 1. Local lesions on tobacco caused by Lily strain (25 days).
Fig. 2. Diseased tulip, variety Zimmerman's Triumph.
Fig. 3. Tobacco leaf 7 days after inoculation with the tulip strain, 11 days after left-hand half of leaf (*b*) inoculated with type *cucumber virus* 1.
Fig. 4. Local lesions on cucumber caused by tulip strain (19 days).

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REVIEWS

Forest Pathology. By J. S. Boyce. Pp. x + 600. London: McGraw-Hill Publishing Co., Ltd. 1938. 30*s.* 0*d.*

Following a brief introductory chapter, Ch. II contains a short discussion of the nature, symptoms and terminology of plant disease, and Ch. III a brief outline of the fungi based on Heald's presentation but emphasizing the Basidiomycetes. Ch. IV deals shortly with the non-infectious diseases of trees, the author remarking that "few of them have been carefully studied" and "the aggregate damage they cause to the forests of this country is not great". Ch. V is devoted to seedling diseases and Ch. VI to root diseases—including mycorrhiza and root nodules. Chs. VII and VIII deal, respectively, with foliage diseases of hardwoods and of conifers. Chs. IX–XVII are devoted to stem diseases as follows: Rusts of conifers; White pine blister rust; Cankers of conifers; Cankers of hardwoods; Galls and witches' brooms; Diebacks and wilts; Mistletoes, epiphytes and climbers; Decay; Rots. Chs. XVIII–XX are concerned with deterioration of forest products: decay in timber; decay in wood; sap stains. Ch. XXI outlines the general principles of forest-disease control. Appendix A contains brief directions for making fungicides and Appendix B a list of common and scientific names of trees. Terminating the chapters are useful bibliographies, and the book concludes with a good index.

The only comparable recent work in English is Hubert's *Outline of Forest Pathology*, 1931, which deals with the same range of material, and is of approximately equal size and calibre. The two volumes resemble each other in their general treatment of introductory matter and of timber and wood decay, but differ in the arrangement of the parasitic diseases of trees. Prof. Boyce arranges his material on the basis of the parts of the tree affected and the tree species, whilst Prof. Hubert bases his arrangement on the taxonomic sequence of the causal organisms, prefacing his consideration by detailed keys of symptoms. Prof. Boyce states that his experience has convinced him that his method is the better, and gives his reasons, whilst Prof. Hubert would, undoubtedly make an equally good case.

A similar problem faces anyone who attempts to teach plant pathology. My own experience has been with students of agriculture and horticulture, and I have tried out the two systems alternately over a number of years. I have come definitely to the conclusion that the arrangement of material by taxonomic sequence of parasites, combined with summarizing reviews of diseases arranged on the plant organ and host basis, is the better. The ordering of material in this way affords not only a more logical presentation of the subject but appears to be much more convincing to students, even those of essentially practical type. The danger is that such courses may become more mycological than pathological in outlook. The organ and host arrangement, in my experience, tends to lead to an empirical and rule of thumb attitude on the part of students, whereas the taxonomic arrangement is equally successful as a method of presenting data and yet emphasizes principles. In the study of plant disease, the essential thing is not a knowledge of a great number of empirical data, but a knowledge of pertinent data, combined with an understanding of principles and a capacity to adapt these to particular cases.

Dr Boyce has compiled and selected his material very conscientiously and, within the general plan, the detailed arrangement is excellent. The text is clear and simple as befits a text-book, but, although it is difficult to say just why, the author's style of writing is curiously dull and uninspired. There are quantities of sentences such as "Fall sowing in northern nurseries where the soil remains practically continuously frozen is less liable to result in loss than spring sowing, although weather is so variable as to time that this method of control is somewhat uncertain" or "Probably soil

character has been affected by the elimination of chestnut, but whether favourably or unfavourably it is difficult to surmise". Having read pages of this sort of thing one feels quite cheered by coming across so charming a name as "the weeping conk", such a sequence as "paintbrush, lousewort, owl's-clover, and bird's beak", or such a delicious sentence as "Individual witches' brooms also die frequently".

The book is illustrated by 216 text-figures, many of which are original, and few of which state the magnification. The only misprint noted is in the legend to Text-fig. 202.

WILLIAM B. BRIERLEY.

Yearbook of Agriculture, 1937. Pp. vi + 1497. United States Department of Agriculture, Washington, D.C. $2·00.

This volume concludes the survey of animal and plant improvement begun in the Yearbook, 1936 (see *Ann. appl. Biol.* 1937, **24**, 679), which dealt with the genetics and breeding of cereals, sugar plants, fibre plants, tobacco, cattle, swine, sheep, horses and mules, and poultry.

The *Yearbook*, 1937, covers an enormous and varied field dealing with: Vegetables—general (5 pp., Boswell); Tomato, Pepper, and Eggplant (31 pp., Boswell); Onions (26 pp., H. A. Jones); Peas and Beans (32 pp., Wade); Leafy cruciferous vegetables (17 pp., Magruder); Root vegetables (26 pp., Poole); Salad crops (14 pp., Thompson); Vegetable crops—appendix (39 pp., Boswell); Sweet corn (16 pp., Poole); Popcorn (10 pp., Brunson); Potatoes (40 pp., Stevenson & Clark); Strawberry (51 pp., Darrow); Blackberry and Raspberry (38 pp., Darrow); Currants and Gooseberries (11 pp., Darrow); *Actinidia* spp., *Viburnum* spp., *Elaeagnus* spp., Quince, *Prunus* spp. (14 pp., Darrow & Yerkes); Blueberry (16 pp., Coville); Apple (40 pp., Magness); Pear (16 pp., Magness); Grape (34 pp., Snyder); Stone fruits (84 pp., Cullinan); Subtropical fruits, *Citrus* (78 pp., Traub & Robinson); Nuts (63 pp., Crane, Reed & Wood); Flowers (109 pp., Emsweller, P. Brierley, Lumsden & Mulford); Miscellaneous forage and cover crop legumes (33 pp., McKee & Pieters); Miscellaneous grasses (71 pp., Vinall & Hein); Timothy (18 pp., Evans); Alfalfa (32 pp., Tysdal & Westover); Soybean (36 pp., Morse & Cartter); Clover (25 pp., Pieters & Hollowell); Hop (27 pp., D. C. Smith); Forest trees (38 pp., Schreiner); Angora goats (14 pp., Lambert); Milk goats (21 pp., Simmons & Lambert); Dogs (35 pp., Dawson); Turkeys (17 pp., Marsden & Knox); Ducks (12 pp., Lee); Fur animals (17 pp., Ashbrook); Bees (23 pp., Nolan). There are also the following more general articles: Fundamentals of heredity for breeders (31 pp., Bressman & Hambidge); Vegetative reproduction (7 pp., Magness); Chronology of genetics (22 pp., Cook). The volume opens with a useful summary of the articles, "What the book is about" (52 pp., Hambidge) and concludes with a good index. Certain other papers and extensive bibliographies, which complete and round off the survey, have been excluded by lack of space, but are stated to be available as *Yearbook* separates.

Together, the genetical portions of the two *Yearbooks* total 2450 well-illustrated pages, containing 69 articles, and they form by far the most comprehensive treatise on animal and plant breeding yet published in the English language. Nothing is of more vital concern to farmers and growers than the improvement of plants and animals, or more valuable to students than an authoritative source book of viewpoints and data. To write for both practical farmers and students is an exceedingly difficult task, but most of the authors have succeeded in presenting their material in reasonably non-technical language which is yet accurate in form and substance.

The articles themselves vary considerably in quality but, on the whole, they are complete and up-to-date. They are written by Americans for Americans and, thus, deal primarily with animals and plants important in American farming, but the United States comprises so many and varied conditions that the volumes are international in content and outlook. Bibliographies citing work referred to in the text are usually a fair guide to the writers' width of view. The bibliography to, for example, Stevenson & Clark's paper on potato breeding, cites 26 American works, 16 British, 13 Russian, 13 German, 2 Scandinavian, 2 Japanese, and 2 French; whilst that to

e.g. Dawson's paper on heredity in the dog, cites 22 American works, 18 British, 9 German, 5 Scandinavian, 4 Russian, 3 French, 2 Swiss, and 1 each Dutch, Polish, Italian, and Japanese; and these bibliographies are typical.

The volumes contain not only an account of what has been done and is being done to improve plants and animals in the U.S.A. They are, also, an attempt to make a frank appraisal of the present situation—not only to sum up achievements, but to expose weaknesses and shortcomings. The achievements of American breeders are magnificent and, because of them, entire fields of American agriculture have been remodelled. There are, however, regions of agricultural practice where modern genetical knowledge and technique are still largely unapplied or where breeding is, at best, largely empirical. In writing of such problems the authors consider the possibilities and have tried to foresee fruitful lines of enquiry.

The survey was organized by a Committee on Genetics appointed in 1933 by the U.S. Secretary of Agriculture, Mr Henry A. Wallace, himself not only a distinguished public servant but a plant breeder for some 30 years. To each volume he has contributed a foreword and extracts from these may fittingly close this notice. "I trust that the day will come when humanity will take as great an interest in the creation of superior forms of life as it has taken in past years in the perfection of superior forms of machinery. In the long run superior life forms may prove to have a greater profit for mankind than machinery". "Man's control of his future may depend in the long run on whether his biological knowledge, which is constructive, can catch up with his knowledge of the physical sciences, which has taught him so much about how to destroy".

WILLIAM B. BRIERLEY.

Physiological Genetics. By R. Goldschmidt. Pp. ix+375. London: McGraw-Hill Publishing Co., Ltd. 1938. 24*s.* 0*d.*

The author has endeavoured "to present the entire material available", and "to organize it into the skeleton of a future science of physiological genetics". The book is written in a technical and somewhat involved style, and knowledge of genetics and embryology is assumed on the part of the reader.

The work is arranged in four sections, and terminates with a useful bibliography of 45 pages, and author and subject indexes. Section I is an introductory note contrasting static genetics with dynamic or physiological genetics.

Section II (260 pages) contains a detailed consideration of the mutated gene and the potentialities of development. The general thesis underlying the author's treatment is that an understanding of the action of the gene can only be obtained by comparing the effects of the mutated gene upon development with effects produced by external agencies upon the development of the Wild type. Incorporating a wealth of data from plant and animal studies, the author builds up a coherent and logical scheme of the genetics of development processes based on the concept of genes as affecting rates. An interesting omission is any reference to Garrod's work.

Section III (20 pages) deals with the role of the cytoplasm in heredity. The author concludes that whilst the cytoplasm is the substratum for genic action, and its specificity one of the prerequisites of orderly development, no specific hereditary traits exist that are transmitted through the cytoplasm and are individually caused by a genetic property of the cytoplasm. Plant plastids are regarded as a third independent constituent of the cell in regard to heredity.

In Section II the existence of the gene is taken for granted, since the data can be considered independently of conceptions regarding its nature. In Section IV (35 pages), the nature of the gene is discussed and, to readers who do not specialize in genetics, these pages will be as interesting as they are controversial. In Prof. Goldschmidt's view "The developments in the last years of genetical research have now led to a point where it has become necessary to ask the radical question: Is not the whole conception of the gene as a hereditary unit obsolete?" He answers this question in the affirmative, considering that the evidence strongly suggests "That the chromosome is the actual hereditary unit controlling the development of the Wild type, that

purely steric changes at the individual points of its length produce deviations from the Wild type which may be described as mutations, even as point mutations, though no actual Wild type allelomorph and therefore no gene exists." This means, more simply, that chromosomes are physiological wholes, and that genes are spatial internal rearrangements of parts of them, which alter their functions. To replace the gene theory the author postulates a chemical theory of heredity, supporting this by the work of Bergmann, Koltzoff, Wrinch, etc., and remarks "It remains for the physico-chemist to decide whether or not the new model could also take care of the independent catalytic actions of what was considered to be a gene."

WILLIAM B. BRIERLEY.

The Study of Heredity. By E. B. Ford. Pp. 256. London: Thornton Butterworth. (Home University Library, Vol. 187.) 1938. 2*s.* 6*d.*

This book is a first-class introduction to the subject for any intelligent person who will apply himself seriously to its study. The chapters run: The physical basis of heredity; The laws of inheritance; The inheritance of sex; Mutation; The nature of heredity; Variation; The action of genetic factors; Selection; Practical applications, and human heredity; Evolution. The book closes with a glossary, a bibliography of sixteen annotated references, and an index.

The author writes in a clear but condensed style, examples are drawn primarily from the animal kingdom, and his treatment of the subject has been influenced largely by the work and views of Darlington, Fisher, Haldane, and Huxley. In discussing human heredity the author makes statements which might not be accepted by all workers. The book is so up-to-date that, even four months after its publication, two of the volumes cited in the bibliography have not yet appeared. Among the other books suggested for further reading are one or two which are perhaps rather out-of-date, one which is very stiff going for first-class Honours students, and one which contains whole paragraphs admitted by professors of the subject to be unintelligible.

WILLIAM B. BRIERLEY.

Tropical Fruits and Vegetables: an account of their Storage and Transport. By C. W. Wardlaw. Pp. xii+224. Imperial College of Tropical Agriculture, Trinidad, B.W.I.; Low Temperature Research Station, Mem. No. VII. 1937. 4*s.* 0*d.*

This bulletin comprises articles, originally published in *Tropical Agriculture*, 1937, 14, nos. 3–12, dealing with the storage and transport of some seventy-four fruits and vegetables indigenous to, or capable of being grown in, the tropics. Attention is chiefly restricted to the major practical issues. The arrangement is alphabetical under the common names of the plants, and to each is appended a good bibliography. A rather unexpected thing is the number of plants included which are usually regarded as being crops of temperate or warm temperate lands: beans and peas, beetroot, various common Brassicas, carrot, celery, filbert, lettuce, radish, spinach, water-cress. Tropical crops, naturally, receive the greatest consideration, special attention being given to avocado, banana, *Citrus*, mango, onion, papaw, persimmon, pineapple, potato, sweet potato, tomato.

The tomato may be taken as an example of the author's treatment: the fruit is discussed in 26 pages under the following heads: Introductory; General considerations; Chemical changes during development and ripening; Pre-storage factors and quality (colour development, effects of fertilizers and soil moisture, temperature of growth, late harvesting, disease incidence); Harvesting maturity; Debuttoning; Packing-shed treatments (disinfectant treatment, standardization of packing maturity, culling and grading for size and colour, wrapping and packing); Transport by rail; Rapid cooling;

Interruption of cold storage; Storage and ripening temperatures (storage at low and higher temperatures, experience in various countries, storage of tropically grown fruit); Low temperature injury (symptoms of chilling); Loss in weight; Effect of light on ripening; Sulphur dioxide injury; Ethylene ripening; Gas storage; 64 references.

Much of the literature on this subject is scattered in journals which, often, are not easily available and this bulletin, which seems to have been compiled carefully, is a very useful publication.

WILLIAM B. BRIERLEY.

Erosion and Soil Conservation. By G. V. Jacks and R. O. Whyte. *Herbage Publication Series, Bull.* No. 25. Pp. 206. Aberystwyth: Imperial Bureau of Pastures and Forage Crops. (Also published as *Technical Communication* No. 36 from the Imperial Bureau of Soil Science, Harpenden.) 1938. 5*s*.

Soil erosion is one of the most disquieting features in world agriculture today and, in many countries, the capital loss is taking place with a rapidity and on a scale which, in any other basic industry, would lead to immediate national action. Although it has reached almost catastrophic proportions soil erosion is not, like an earthquake, an "act of God": it is an entirely preventible process, the principles of soil conservation are fairly well established and it is imperative that they become widely known and applied before the destruction becomes even more calamitous. A first essential is an accurate survey of the present position, so that conditions and trends and methods of control and regeneration in various lands may be compared. In compiling this account of contemporary soil erosion, the authors have not only consulted widely in the literature of the subject but have had the active cooperation of correspondents and workers throughout the world, and this has enabled them to present a brief but authoritative picture of conditions in the Mediterranean region, U.S.S.R., India, Ceylon, East Indies, China, Japan, French overseas possessions, South Africa, Rhodesia, East Africa, West Africa, U.S.A., Canada, West Indies, Australia, and Fiji. The bulletin contains a mass of information and is well put together but a useful addition would have been a concluding section summarizing and comparing conditions and developments in the various countries. This bulletin should receive the widest circulation possible among not only agricultural scientists but also sociologists and administrators.

WILLIAM B. BRIERLEY.

Sixty Years of Botany in Britain (1875–1935). *Impressions of an Eye-witness.* By F. O. Bower. Pp. vii+112. With frontispiece and 13 other illustrations. London: Macmillan and Co., Ltd. 1938. 10*s*. 6*d*.

Prof. F. O. Bower was born four years before the publication of the *Origin of Species* and, during 60 years bridging the nineteenth and twentieth centuries, he was one of the international figures in botanical science. In that period he came to know intimately most of the men who, with himself, helped to make botanical history.

The autobiographical chapters opening the book give a vivid impression of the conditions of botanical study in the 1870's and 1880's, conditions which would seem hardly credible to modern students who take fully equipped laboratories for granted. In a series of admirable character sketches accompanied by excellent portraits, the author introduces the men who brought about these changes, names familiar to every student of botany. A short and rather more technical chapter gives a comprehensive survey of the changes of outlook on the vegetation of the land during the period since 1875 and these pages, which are really an epitome of Prof. Bower's

life-work, are a masterly piece of writing. In a final chapter the author attempts to relate the botanical events of 60 years to the broad stream of scientific progress.

The illustrations form a magnificent gallery of portraits and one's only regret is that Prof. Bower's modesty has excluded his own.

WILLIAM B. BRIERLEY.

Herbals: Their Origin and Evolution. A Chapter in the History of Botany, 1470–1670. By AGNES ARBER. 2nd edition. Pp. xxiv + 326. Cambridge: University Press. 1938. 21*s.* 0*d.*

Mrs Arber has rewritten and enlarged her volume on *Herbals*, of which the first edition (1912) has long been out of print. The general plan of the book remains unaltered, but newer knowledge has enabled her to fill in many interesting details and to add sections dealing with botany in Spain and Portugal, and with the origin of herbaria. The illustrations have been increased by 5 plates and 18 text-figures. Appendix I has been enlarged, Appendix II trebled, and a third appendix, a subject-index to Appendix II, has been added.

In 1934 Mrs Arber published her magistral work on *The Gramineae*, and in reviewing this (*Ann. appl. Biol.* 1935, **22**, 433), I said: "Permeating the whole book is a certain fragrance, a controlled discursiveness and wide-ranging quality that one always associates with the great herbalists of a bygone age. It is a style of writing almost unique in the professionalized botany of to-day and it is a delight to read." Mrs Arber has now written of the great herbalists themselves. It is a subject she has made peculiarly her own, and in which her sympathetic vision, and her charming and scholarly style find natural expression and full scope. Her book is pure gold. Those who treasured the old edition will find increased delight in the new, although they may regret the passing of the familiar covers, whilst those who now meet the book for the first time have an enviable joy in store.

WILLIAM B. BRIERLEY.

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1935 Duffield, C. A. W., F.R.E.S., The Cooper Technical Bureau, 47, Russell Square, London, W.C. 1.

1923 Du Porte, Ernest Melville, M.Sc., Ph.D., F.R.E.S., F.R.M.S., Macdonald College, Montreal, Canada.

1928 Eastham, Prof. L. E. S., M.A., M.Sc., Department of Zoology, The University, Sheffield.

1927 Edwards, E. E., M.Sc., Department of Agricultural Zoology, University College, Cardiff.

1935 Ekins, Miss E. H., B.Sc., Principal, The College, Studley, Warwickshire.

1934 Engledow, Prof. F. L., M.A., C.M.G., School of Agriculture, Cambridge.

1922 Esdaile, Miss P. C., D.Sc., F.Z.S., King's College of Household and Social Science, Campden Hill Road, London, W. 8.

1935 Evans, A. C., B.Sc., D.I.C., Rothamsted Experimental Station, Harpenden, Herts.

1927 Everett, J., B.A., Canterton Cottage, Lyndhurst, Hants.

1920 Fahmy, T., D.Sc., Mycological Division, Plant Protection Section, Ministry of Agriculture, Giza, Cairo, Egypt.

1920 Fenton, E. Wyllie, M.A., B.Sc., F.R.E.S., Biological Department, Edinburgh, and East of Scotland College of Agriculture, 13, George Square, Edinburgh.

1937 Ferguson, R. G., M.Agr.Sc., Herts. Institute of Agriculture, Oaklands, St Albans, Herts.

1937 Fidler, J. H., B.A., Ph.D., Dept. of Agricultural Entomology, Victoria University, Manchester, 13.

1929 Findlay, W. P. K., M.Sc., A.R.C.S., D.I.C., Forest Products Research Laboratory, Princes Risborough, Bucks. (Botanical Secretary, 1936, General and Botanical Secretary, 1937– .)

1935 Fish, S., Department of Agriculture for Victoria, Public Offices, Melbourne, Victoria, Australia.

1919 Fisher, K., The School, Oundle, Northampton.

1923 Fisher, R. C., B.Sc., Ph.D., Forest Products Research Laboratory, Princes Risborough, Bucks. (Vice-President, 1936; Council, 1934–1936.)

1931 Foister, C. E., B.A., Ph.D., Royal Botanic Garden, Edinburgh.

1920 Fox-Wilson, G., N.D.Hort., F.L.S., F.R.E.S., R.H.S. Laboratories, Wisley, Ripley, Surrey. (General and Zoological Secretary, 1935–36; Zoological Secretary, 1933–1934; 1937– ; Council, 1927–1929.)

1936 Franklin, Miss M. T., B.Sc., Institute of Agricultural Parasitology, Winches Farm, St Albans, Herts.

1913 Fryer, J. C. F., O.B.E., M.A., F.R.E.S., Pathological Laboratory, Milton Road, Harpenden, Herts. (President, 1926–1927; Treasurer, 1914–1919; Council, 1921, 1924–1925.)

1933 Gadd, C. H., D.Sc., Tea Research Institute, St Coombs, Talawakelle, Ceylon.

1930 Galloway, L. D., M.A., 31 Lake Close, Wimbledon, S.W. 19.

1935 Garrett, S. D., M.A., Rothamsted Experimental Station, Harpenden, Herts.

1936 Gibbons, J., M.Sc., Avoncroft, Stoke Prior, Bromsgrove, Worcs.

1927 Gibson, W. H., O.B.E., D.Sc., F.I.C., F.Inst.P., Director, The Linen Industry Research Association, The Research Institute, Lambeg, Co. Antrim, Northern Ireland.

1938 Gill, N. T., B.Sc., Ph.D., Midland Agricultural College, Sutton Bonington, Loughborough.

1937 Gillespie, J., B.Sc., Botany Dept., The University, Reading.

1937 Gillespy, T. G., B.Sc., A.R.C.S., University of Bristol Fruit and Vegetable Preservation Research Station, Campden, Glos.

1920 Gimingham, C. T., O.B.E., F.I.C., F.R.E.S., Pathological Laboratory, Milton Road, Harpenden, Herts. (Zoological Editor, 1934– ; Council, 1929–1932; Vice-President, 1937; President, 1938– .)

1920 Glynne, Miss M. D., M.Sc., F.L.S., Rothamsted Experimental Station, Harpenden, Herts.

1920 Goodey, T., D.Sc., Institute of Agricultural Parasitology, Winches Farm, Hatfield Road, St Albans. (President, 1934–1935; Council, 1923–1929; 1936–1938.)

1929 Grainger, J., Ph.D., Tolson Memorial Museum, Ravensknowle, Huddersfield.

1921 Gray, Prof. P. H. H., M.A., Macdonald College, Quebec, Canada.

1929 Green, D. E., M.Sc., R.H.S. Laboratories, Wisley, Ripley, Surrey.

1935 Green, T. L., Dept. of Zoology, The University, Bristol.

1933 Gregor, J. W., Ph.D., Scottish Plant Breeding Station, Craigs House, Corstorphine, Edinburgh.

1934 Grieve, B. J., M.Sc., Ph.D., D.I.C., Botany School, University of Melbourne, Victoria, Australia.

1921 Grubb, N. H., M.Sc., East Malling Research Station, East Malling, Kent.

1934 Güssow, H. T., F.L.S., F.R.M.S., Dominion Botanist, Central Experimental Farm, Ottawa, Canada.

1920 Gwynne-Vaughan, Prof. Dame Helen, D.B.E., D.Sc., LL.D., F.L.S., Birkbeck College, Chancery Lane, London, E.C. 4.

1920 Halket, Miss A. C., B.Sc., Bedford College, Regent's Park, London, N.W.

1930 Hall, W. J., D.Sc., A.R.C.S., F.R.E.S., Director, Citrus Experimental Station, P.O. Mazoe, S. Rhodesia, Africa.

1924 Harris, R. V., A.R.C.S., B.Sc., East Malling Research Station, East Malling, Kent.

1936 Hatt, W. E., Garden Fertilisers Ltd., Nelson Road, Wanstead, London, E. 11.

1938 Harvey, G. L., Asiatic Petroleum Co. Ltd., Great St Helens, London, E.C. 3.

1927 Hatton, R. G., C.B.E., M.A., D.Sc., Director, East Malling Research Station, East Malling, Kent. (Council, 1928.)

1938 Henderson, F. Y., D.Sc., Imperial College of Science, London, S.W. 7.

1935 Herford, Miss G. M., B.Sc., 1, Frognal Gardens, Hampstead, N.W. 3.

1933 Hey, G. L., M.A., c/o The Murphy Chemical Co., Wheathampstead, Herts.

1920 Hill, Sir A. W., K.C.M.G., M.A., Sc.D., F.R.S., F.L.S., Director, Royal Botanic Gardens, Kew, Surrey.

1920 Hiscox, Miss E. R., B.Sc., National Institute for Research in Dairying, Shinfield, Nr. Reading.

1936 Hobson, R. P., B.Sc., Ph.D., School of Agriculture, University College of North Wales, Bangor.

1924 Hockey, J. F., B.S.A., Pathologist in Charge, Plant Pathology Laboratory, Kentville, Nova Scotia, Canada.

1935 Hodson, W. E. H., A.R.C.S., D.I.C., The University, Reading.

1937 Holborn, G. M., B.Sc., A.R.C.S., Jeyes Sanitary Compounds, Richmond Street, Plaistow, London, E. 13.

1938 Holman, H. J., B.Sc., A.R.C.S., A.I.C., Imperial Institute, South Kensington, London, S.W. 7.

1919 Horne, A. S., D.Sc., F.L.S., F.G.S., Botany School, Imperial College of Science, London, S.W. 7.

1920 Horton, E., B.Sc., F.I.C., 10, Crieff Road, Wandsworth Common, London, S.W. 18.

1927 Howes, F. N., D.Sc., Royal Botanic Gardens, Kew, Surrey. (Council, 1930–1931.)

1928 Hughes, A. W. McKenny, D.I.C., Natural History Museum, S. Kensington, London, S.W. 7.

1936 Hull, R., B.Sc., A.R.C.S., Midland Agricultural College, Sutton Bonington, Loughborough.

1938 Hunter-Smith, M. B. E., B.Sc., N.D.A., Herts Institute of Agriculture, Oaklands, St Albans.

Orig. Imms, A. D., M.A., D.Sc., F.R.S., F.L.S., F.R.E.S., Zoological Laboratory, The Museums, Cambridge. (President, 1930–1931; Vice-President, 1924–1927; Treasurer, 1920–1930; Council, 1914–1920; 1933–1935.)

1918 Jackson, Miss D. J., F.L.S., F.R.E.S., North Cliff, St Andrews, Fife, Scotland.

1927 Jacobs, S. E., Ph.D., Bacteriology Department, Imperial College of Science, London, S.W. 7.

1927 James, H. C., D.Sc., Zoological Laboratory, The Museums, Cambridge.

1927 Jary, S. G., B.A., S.E. Agricultural College, Wye, Kent. (Council, 1934-1936.)

1934 Johnson, C. G., B.Sc., London School of Tropical Medicine and Hygiene, Gower Street, London.

1938 Johnson, L. R., M.Sc., Department of Agriculture, The University, Leeds 2.

1931 Jones, A. P., M.Sc., Department of Agriculture, The University, Leeds 2.

1934 Jones, E. Parry, B.Sc., Ph.D., A.I.C.T.A., F.R.E.S., Citrus Experimental Station, P.O. Mazoe, S. Rhodesia, Africa.
1927 Jones, G. H., M.A., Plant Protection Section, Ministry of Agriculture, Cairo, Egypt.
1927 Joseph, E. G., B.Sc., Redcot, Swanley, Kent.
1935 Kearns, H. G. H., B.Sc., Ph.D., The Research Station, Long Ashton, Bristol.
1932 Kerr, H. W., Ph.D., M.Sc., Director, Bureau of Sugar Experiment Station, Brisbane, Australia.
1920 Kidd, F., M.A., D.Sc., Director Low Temperature Research Station, Downing Street, Cambridge.
1907 King, H. H., F.L.S., F.R.E.S., Government Entomologist, Wellcome Tropical Research Laboratories, Khartoum, Sudan.
1907 King, Prof. L. A. L., M.A., West of Scotland Agricultural College, 6, Blythewood Square, Glasgow.
1926 Kingston, H. T., Athlone, High Road, Broxbourne, Herts.
1933 Kramer, L. M. J., M.A., Ph.D., City of London School, Victoria Embankment, London, E.C. 4.
1921 Lacey, Miss M. S., M.Sc., Botanical Department, Imperial College of Science, London, S.W. 7.
1935 Ladell, W. R. S., F.I.C., Amity Hall, Alley, P.O. Jamaica, B.W.I.
1921 Laurie, Prof. R. D., M.A., F.Z.S., Department of Zoology, University College of North Wales, Aberystwyth.
1933 Leach, R., B.A., School of Agriculture, Cambridge.
1934 Lean, O. B., B.Sc., D.I.C., Imperial Chemical Industries, Jealott's Hill, Bracknell, Berks.
1926 Le Pelley, R. H., Ph.D., Department of Agriculture, Nairobi, Kenya, Africa.
1920 Lloyd, Llewellyn, D.Sc., Zoological Department, The University, Leeds.
1937 Lovibond, Miss C. B. J., B.Sc., Rothamsted Experimental Station, Harpenden, Herts.
1931 McClean, A. P. D., D.Sc., Natal Herbarium, Berea, Durban, Natal, Union of S. Africa.
1914 McClellan, F. C., C.B.E., M.R.A.C., F.L.S., Director of Agriculture, Zanzibar.
1936 Macdonald, J. A., B.Sc., Ph.D., The University, St Andrews.
Orig. Macdougall*, Prof. R. S., M.A., D.Sc., F.R.S.E., F.R.E.S., Ivy Lodge, Gullane, E. Lothian, Scotland. (Vice-President, 1914–1919; Council, 1908–1913.)
1929 MacGill, Miss E. I., D.Sc., Linkfield, Hawthorn Road, Denton, Manchester.
1927 McLennan, Miss Ethel, D.Sc., Botanical Department, The University, Melbourne, Australia.
1925 Macleod, D. J., M.A., 20 Highworth Avenue, Cambridge.
1938 McLeod, J., D.Sc., Cooper Field Research Station, Little Gaddesden, Herts.
1909 Mangan, Prof. J., M.A., 3, Ceiriog Close, Penarth, Glamorgan, S. Wales.
1920 Mangham, Prof. S., M.A., University College, Southampton.
1917 Mann, H. H., D.Sc., F.L.S., Woburn Experimental Station, Aspley Guise, Bedfordshire.
1938 Marsh, R. W., M.A., Research Station, Long Ashton, Bristol.
1934 Marshall, J., B.Sc., A.R.C.S., A.I.C.T.A., c/o Empire Cotton Growing Corporation, King's Buildings, Dean Stanley Street, London, S.W. 1.

1930 Martin, H., D.Sc., A.R.C.S., Research Station, Long Ashton, Bristol. (Council, 1936–1938; Vice-President, 1938.)

1922 Mason, E. W., M.A., M.Sc., Imperial Mycological Institute, Kew, Surrey.

1920 Mason, F.A., F.R.M.S., 29, Frankland Terrace, Leopold Street, Leeds.

1933 Mason, Miss M., B.Sc., (Hort.), Ministry of Agriculture and Fisheries, 10 Whitehall Place, London, S.W. 1.

1927 Massee, A. M., D.Sc., F.R.E.S., East Malling Research Station, East Malling, Kent. (Council, 1932–1934.)

1920 Mattick, A. T. R., B.Sc., Ph.D., National Institute for Research in Dairying, Shinfield, Nr. Reading.

1938 Meggitt, F. J., M.Sc., Ph.D., University College, Rangoon.

1933 Meiklejohn, Miss J., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.

1932 Metcalfe, Miss M. E., B.Sc., Ph.D., 20, Llantwit Road, Treforest, Glamorganshire.

1923 Miles, H. W., D.Sc., Ph.D., Department of Agricultural Entomology, Victoria University, Manchester. (Council 1937– .)

1921 Millard, W. A., D.Sc., Department of Agriculture, The University, Leeds 2.

1932 Misra, A. B., D.Sc., Ph.D., F.Z.A., F.R.E.S., Department of Zoology, Benares Hindu University, India.

1938 Moore, E. J., M.Sc., Central Technical College, Suffolk Street, Birmingham, 1.

1932 Moore, Miss E. S., B.Sc., Ph.D., Mycologist, Tobacco Research Laboratory, Balfour, C.P. South Africa.

1928 Moore, W. C., M.A., Plant Pathological Laboratory, Milton Road, Harpenden, Herts. (Council 1938– .)

1922 Morland, D. M. T., M.A., Rothamsted Experimental Station, Harpenden, Herts.

1920 Morris, H. M., M.Sc., Agricultural Department, Nicosia, Cyprus.

1920 Mosley, F. O., F.L.S., Pathology Laboratory, Messrs Lowe and Shawyer, Uxbridge, Middlesex.

1914 Munro, Prof. J. W., M.A., D.Sc., Zoology Department, Imperial College of Science, London, S.W. 7. (Vice-President, 1933; General and Zoological Secretary, 1928–1932; Zoological Editor, 1933; Council, 1922–1924, 1927.)

1919 Murphy, A. J., Technical Chemist, The Murphy Chemical Co., Wheathampstead, Herts.

1920 Murphy, Prof. P. A., Sc.D., B.A., Albert Agricultural College, Glasnevin, Dublin, Irish Free State.

1938 Musgrave, A. J., B.Sc., D.I.C., Imperial College of Science and Technology, London, S.W. 7.

1925 Muskett, A. E., B.Sc., A.R.C.S., D.Sc., The Queen's University, Belfast, N. Ireland.

1937 Narasimhan, M. J., B.A., Mycologist, Dept. Agriculture, Bangalore Mysore State, India.

1937 Nattrass, R. M., Ph.D., Dept. of Agriculture, Nairobi, East Africa. Newport, Salop. (Council 1937– .)

1928 Nel, R. I., B.Sc., M.Sc., Naga Hoeta Estate, Pematang Siantar, Sumatra, (East Coast).

1928 Newton, H. C. F., Ph.D., A.R.C.S., D.I.C., Harper Adams Agricultural College, Newport, Salop. (Council, 1937–)

1930 Norman, A. C., D.Sc., F.I.C., Sails Dept., Iowa State College, Ames, Iowa, U.S.A.

1921 Nowell, Wm, D.I.C., F.L.S., Director, East African Agricultural Research Institute, Amani, Tanga, Tanganyika Territory, E. Africa.

1928 Nutman, F. J., B.Sc., A.R.C.S., East African Agricultural Research Station, Amani, Tanga, Tanganyika Territory, E. Africa.

1923 Ogilvie, L., M.A., M.Sc., Research Station, Long Ashton, Bristol.

1925 Oldham, J. N., B.Sc., Ph.D., "Heriots", 336 Hatfield Road, St Albans, Herts.

1937 Oyler, Miss Enid, B.Sc., Experimental and Research Station, Cheshunt, Herts.

1928 Parkin, E. A., B.Sc., Ph.D., Forest Products Research Laboratory, Princes Risborough, Bucks.

1935 Pearl, R. T., B.Sc., A.R.C.S., South Eastern Agricultural College, Wye, Kent.

1914 Petherbridge, F. R., M.A., School of Agriculture, Cambridge.

Orig. Pethybridge, G. H., M.A., M.R.I.A., Ph.D., B.Sc., Pathological Laboratory, Milton Road, Harpenden, Herts. (Vice-President, 1926–1927; Council, 1912–1913, 1925–1927.)

1928 Pickles, A., B.Sc., Imperial College of Tropical Agriculture, Trinidad, B.W. Indies.

1915 Porter*, A., D.Sc., Department of Zoology, McGill University, Montreal, Canada.

1934 Potter, C., B.Sc., Rothamsted Experimental Station, Harpenden, Herts.

1907 Poulton*, Prof. Sir Edward B., M.A., D.Sc., LL.D., F.R.S., Wykeham House, Banbury Road, Oxford. (President, 1922–1923; Vice-President, 1909–1913.)

1938 Powell, A. K., M.Sc., Imperial College of Science, London, S.W. 7.

1919 Prain*, Sir David, Lt.-Col., C.M.G., C.I.E., M.A., M.B., F.R.S., LL.D., F.R.S.E., V.M.H., The Well Farm, Warlingham, Surrey. (President, 1919–1921.)

1934 Pratt, A., B.Sc., Bell-Baxter School, Cupar, Fife.

1923 Preston, N. C., B.Sc., Harper Adams Agricultural College, Newport, Salop.

1928 Ramsbottom, J., O.B.E., M.A., Dr.Sc., F.L.S., Keeper of Botany, British Museum (Natural History), Cromwell Road, London, S.W. 7.

1931 Reichert, I., Ph.D., Chief Plant Pathologist, P.O. Box 15, Rehoboth, Palestine.

1921 Richards, E. H., B.Sc., F.I.C., Rothamsted Experimental Station, Harpenden, Herts.

1928 Richards, O. W., M.A., D.Sc., 29*a*, Edith Road, London, W. 14.

1936 Riley, N.D., Keeper of Entomology, British Museum (Natural History), London, S.W. 7.

1921 Roach, W. A., D.Sc., A.R.C.S., D.I.C., A.I.C., East Malling Research Station, East Malling, Kent. (Council, 1935–1936.)

1914 Roberts, A. W. Rymer, M.A., F.R.E.S., Molteno Institute for Research in Parasitology, Cambridge.

1923 Robinson, D. H., B.Sc., Harper Adams Agricultural College, Newport, Salop.

1918 Robson, R., Institute of Agriculture, Chelmsford.

1920 Roebuck, A., N.D.A., Midland Agricultural College, Sutton Bonnington, Loughborough. (Council 1938– .)

1929 Rogers, W. S., B.A., East Malling Research Station, East Malling, Kent.

1919 Russell, Sir E. John, D.Sc., F.R.S., Director, Rothamsted Experimental Station, Harpenden, Herts. (Vice-President, 1923; Council, 1921–1923.)

1932 Russell, T. A., B.Sc., A.R.C.S., Department of Agriculture, Paget East, Bermuda.

1929 Salaman, R. N., M.D., F.R.S., Director, Potato Virus Research Station, School of Agriculture, Cambridge.

1914 Salmon, Prof. E. S., F.L.S., S.E. Agricultural College, Wye, Kent. (Council, 1916, 1924–1926.)

1935 Sampson, Miss K., M.Sc., Agricultural Buildings, University College of Wales, Aberystwyth.

1923 Samuel, G., B.Sc., Pathological Laboratory, Milton Road, Harpenden, Herts. (Council 1935–1937.)

1921 Sargent, R. H., Danescroft, Cleveland Avenue, Darlington.

1933 Schimmer, Miss F. C., M.Sc., A.R.C.S., D.I.C., Horticultural College, Swanley, Kent.

1919 Searle, G. O., B.Sc., Linen Industry Research Association, The Research Institute, Lambeg, Co. Antrim, N. Ireland.

1932 Sharga, U. S., M.Sc., Ph.D., Agricultural Dept., Etawah, U.P., India.

1938 Shaw, H., M.Sc., Ph.D., East Malling Research Station, East Malling, Kent.

1932 Sheffield, Miss F. M. L., Ph.D., F.L.S., Rothamsted Experimental Station, Harpenden, Herts.

1936 Sian, W. E., 11 Grove Road, South Woodford, London, E. 18.

1920 Small, Prof. J., D.Sc., F.L.S., Queen's University, Belfast, N. Ireland.

1928 Small, T., M.Sc., A.R.C.S., The States Experimental Farm, Trinity, Jersey.

1920 Smith, E. Holmes, B.Sc., Botany School, Victoria University, Manchester.

1919 Smith, J, Henderson, M.B., Ch.B., B.A., Department of Plant Pathology, Rothamsted Experimental Station, Harpenden, Herts. (Treasurer, 1931– ; President, 1936–1937.)

1920 Smith, Kenneth M., D.Sc., Ph.D., F.R.S., Potato Virus Research Station, School of Agriculture, Cambridge.

1927 Smith, Prof. N. J. G., M.A., B.Sc., Ph.D., Rhodes University College, Grahamstown (Cape), S. Africa.

1913 South, F. W., M.A., 73 Fairway, Aldwick Bay Estate, Bognor Regis, Sussex.

1919 Speyer, E. R., M.A., Experimental and Research Station, Cheshunt, Herts. (Council, 1931–1933; 1937– .)

1920 Spinks, G. T., M.A., Research Station, Long Ashton, Bristol.

1920 Stapledon, Prof. R. G., C.B.E., M.A., Director, Imperial Bureau of Plant Genetics, Agricultural Buildings, Alexandra Road, Aberystwyth.

1933 Steer, W., Danelaugh, London Road, Leybourne, West Malling, Kent.

1925 Stell, F., Department of Agriculture, Port of Spain, Trinidad, British W. Indies.

1936 Steven, W. F., M.A., 110 Church Road, Richmond, Surrey.

1922 Stirrup, H. H., M.Sc., Midland Agricultural College, Sutton Bonnington, Loughborough.

1919 Stone, H., c/o Messrs Benton and Stone, Bracebridge Street, Birmingham.
1926 Storey, H. H., M.A., Ph.D., East African Agricultural Research Station, Amani, Tanga, Tanganyika Territory, E. Africa.
1927 Stoughton, Prof. R. H., D.Sc., A.R.C.S., The University, Reading, Berks. (General and Botanical Secretary, 1933–1934; Botanical Secretary, 1935.)
1933 Sutton, M. A. F., F.L.S., Sutton and Sons, Ltd., Reading, Berks.
1919 Tabor, Prof. R. J., B.Sc., Botany School, Imperial College of Science, London, S.W. 7.
1935 Tarr, H. L. A., Ph.D., Fisheries Research Board, Ottawa, Canada.
1921 Tattersfield, F., D.Sc., F.I.C., Rothamsted Experimental Station, Harpenden, Herts.
1935 Taylor, C. B., B.S.A., Ph.D., Division of Bacteriology, Department of Agriculture, Central Experimental Farm, Ottawa, Canada.
1914 Taylor, H. V., O.B.E., D.Sc., A.R.C.S., Commissioner for Horticulture, Ministry of Agriculture and Fisheries, 10, Whitehall Place, London, S.W. 1. (Council, 1927–1928.)
1936 Templeman, W. G., M.Sc., Imperial Chemical Industries, Jealott's Hill Research Station, Bracknell, Berks.
1927 Thaysen, A. C., Ph.D., 3, Cumberland Court, 83, West Hill, S.W. 15. (Council, 1933–1935.)
1931 Thomas, I., M.Sc., School of Agriculture, University College of N. Wales, Memorial Buildings, Bangor.
1938 Thompson, H. W., M.Sc., Department of Agriculture, The University, Leeds, 2.
1928 Thompson, W. R., F.R.S., D.Sc., Imperial Institute of Entomology, Farnham House Laboratory, Farnham Royal, Bucks. (Vice-President, 1930–1932.)
1933 Thomson, J. R., B.Sc., School of Agriculture, Khartoum North, Sudan. Herts. (Council 1936–1938.)
1919 Thornton, H. G., B.A., D.Sc., Rothamsted Experimental Station, Harpenden, Herts. (Council, 1936–1938.)
1933 Thorold, C. A., B.Sc., Department of Agriculture, Nairobi, Kenya.
1934 Thorpe, W. H., M.A., Ph.D., Zoological Laboratory, The Museums, Cambridge.
1927 Tincker, M. A. H., M.A., D.Sc., R.H.S. Laboratories, Wisley, Ripley, Surrey. (Council, 1930–1932.)
1919 Trow, Principal A. H., D.Sc., F.L.S., 9, Clive Crescent, Penarth, Glamorgan.
1915 van der Byl, Prof. P. A., M.A., D.Sc., F.L.S., University of Stellenbosch, Stellenbosch, Union of S. Africa.
1929 Vyvyan, M. C., M.A., East Malling Research Station, East Malling, Kent.
1920 Wakefield, Miss E. M., M.A., F.L.S., Royal Botanic Gardens, Kew, Surrey.
1929 Wakely C. T. N., B.Sc., Africa House, Kingsway, London, W.C. 2.
1932 Waldie, J. S. L., B.Sc., N.D.A., The University, Reading, Berks.
1923 Walkden, H., The Raft, Derbyshire Road, Sale, Cheshire.
1936 Walker, Miss M. M., B.A., The Horticultural College, Swanley, Kent.
1931 Wallace, E. R., B.A., Agricultural Institute, Kirton, nr. Boston, Lincs.
1928 Wallace, G. B., B.Sc., Ph.D., Department of Agriculture, Morogoro, Tanganyika Territory, E. Africa.
1919 Wallace, J. C., M.C., Director, Agricultural Institute, Kirton, nr. Boston, Lincs.

Orig. WARBURTON, C., M.A., Yew Garth, Grantchester, Cambridge. (Council, 1905–1913.)

1919 WARE, W. M., D.Sc., S.E. Agricultural College, Wye, Kent. (Council, 1930–1933.)

1922 WARINGTON, Miss K., D.Sc., Rothamsted Experimental Station, Harpenden, Herts.

1930 WATSON, Mrs M. A., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.

1920 WATT, A. S., Ph.D., B.A., Botany School, Cambridge.

1918 WEST, C., D.Sc., A.R.C.S., D.I.C., F.L.S., Low Temperature Research Station, Downing Street, Cambridge. (Council, 1931.)

1923 WESTON, W. A. R. DILLON, M.A., Ph.D., School of Agriculture, Cambridge.

1937 WHITE, H. L., M.A., Ph.D., D.I.C., Experimental and Research Station, Cheshunt, Herts.

1921 WHITEHEAD, T., D.Sc., A.R.C.S., University College of North Wales, Memorial Buildings, Bangor.

1937 WICKENS, G. M., B.Sc., Ph.D., Tobacco Research Station, P.B. 74 B, Salisbury, S. Rhodesia.

1937 WILKINS, W. H., M.A., D.Phil., F.L.S., Mycology Laboratory, University Department of Botany, Oxford.

1912 WILLIAMS, C. B., M.A., Sc.D., Rothamsted Experimental Station, Harpenden, Herts. (Vice-President, 1934–1935; Council, 1933–1935.)

1930 WILLIAMS, P. H., B.Sc., Experimental and Research Station, Cheshunt, Herts. (Council, 1934–1936.)

1919 WILLIS, J. C., M.A., Sc.D., F.R.S., F.L.S., c/o Empire Cotton Growing Corporation, Kings Buildings, Dean Stanley Street, London, S.W. 1.

1923 WILSON, Miss A. P., A.R.C.S., "Pencraft", Bingham Road, Radcliffe-on-Trent, Notts.

1933 WILSON, A. R., B.Sc., Ph.D., Midland Agricultural College, Sutton Bonington, Loughborough.

1914 WILSON, M., D.Sc., A.R.C.S., Royal Botanic Garden, Edinburgh.

1921 WILTSHIRE, S. P., M.A., D.Sc., Imperial Mycological Institute, Kew, Surrey. (Council, 1926–1929; 1935–1937; Vice-President 1936.)

1932 WOOD, J., A.R.C.S., D.I.C., Agricultural Institute, Kirton, nr. Boston, Lincs.

1926 WOODWARD, R. C., M.A., Imperial Chemical Industries, Jealott's Hill Research Station, Bracknell, Berks. (Council 1931–1932.)

1914 WORMALD, H., D.Sc., A.R.C.S., East Malling Research Station, East Malling, Kent. (Council 1936–1938; Vice-President, 1938.)

1936 WORSLEY, R. R. Le G., Ph.D., A.R.C.S., D.I.C., East African Agricultural Research Station, Amani, Tanganyika Territory, East Africa.

1920 WORTLEY, E. J., F.I.C., M.B.E., F.C.S., Director of Agriculture, St Anns, Port of Spain, Trinidad, British West Indies.

LAWS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

I. The Association shall be called "The Association of Applied Biologists."

II. The object of the Association shall be to promote the study and advancement of all branches of Biology with especial reference to their applied aspects.

III. The Association shall consist of Ordinary and Honorary Members.

IV. Each candidate for ordinary membership shall be a subject of the British Crown. The nomination form of each candidate for ordinary membership shall bear the signatures of two members and shall be forwarded to the Secretaries. The nomination shall be submitted to the Council and, if approved, the election of the candidate shall be recommended to the Association at the next General Meeting. For the election of any candidate two-thirds of the votes of the members present and voting shall be cast in favour of the candidate.

V. All ordinary members on first election shall pay an entrance fee of half-a-guinea. Ordinary members shall pay an annual subscription of twenty-five shillings, due on 1 January of each year, or may compound for their subscriptions by payment of a sum of twenty-five pounds.

VI. Every member elected to the Association shall receive notice to that effect from the Secretaries and shall continue a member until his written resignation shall be received by the Secretaries, or until his membership be forfeited under the laws. (A member shall be liable for the annual subscription for the year in which his resignation takes effect and, notwithstanding his resignation, shall, if he so desires, receive any subsequent publications of the Association issued during that year.)

VII. Ordinary members shall be entitled to admission to all the meetings of the Association, to vote thereat, to present papers, to take part in discussions, and to receive a copy of the Association's publications. Each member shall be entitled personally to introduce non-members to any General Meeting of the Association. But no member whose subscription is in arrears shall be entitled to vote at a General Meeting or to receive the Association's publications, nor shall any publication be sent to a new member until his entrance fee and subscription shall have been received.

The Council may remove from the roll of the Association any member whose subscription is one year or more in arrears.

VIII. Honorary Members shall be persons, not subjects of the British Crown, who have contributed to an eminent degree to the advancement of the Science of Applied Biology. They shall be recommended by a majority of the whole Council and elected in the same manner as Ordinary Members. The number of Honorary Members shall not exceed twelve and not more than two shall be elected in any one year.

Honorary Members shall each receive a copy of the Association's publication and shall not be liable for the payment of an entrance fee or annual subscription.

Their privileges shall be the same as those of Ordinary Members except that they shall not be entitled to vote at any election or meeting of the Association.

IX. The business of the Association shall be conducted by a Council consisting of a President, a Treasurer, the Secretaries, of whom there shall be two (one representing the Botanical, the other the Zoological Sections of the Association), the Editors of the Annals, of whom there shall be two, and twelve Ordinary Members. Two members of the Council shall be nominated by the President to act as Vice-Presidents.

X. All properties of the Association, both present and future, shall be deemed to be vested in the Council of the Association for the time being, in conformity with the provisions of the Literary and Scientific Institutions Act, 1854.

XI. The Council shall meet at such times as they may determine; six members shall form a quorum.

XII. The Council shall have the power to fill any vacancies among its number that may occur other than those resulting from the selection for annual retirement from the Council referred to in Law XVII.

XIII. The Council shall have power, at any of their meetings, by two-thirds of the votes of those present and voting, to recommend the removal from the roll of membership of the name of any member for the reason that in their opinion it is contrary to the interests of the Association that he shall remain a member. Such recommendation shall be submitted to the Association at the next General Meeting. For the ejection from the Association of any member two-thirds of the votes of the members present and voting shall be cast in favour of such ejection.

XIV. The Council shall appoint a Publications Committee consisting of the Editors, the Treasurer, two Ordinary Members of the Council, and two Ordinary Members of the Association, who shall be responsible for the publication of the Journal of the Association.

XV. The Council, at a meeting prior to the Annual General Meeting, shall appoint one or more Auditors to audit the Treasurer's accounts.

XVI. The Council shall purchase such books, instruments, specimens, furniture and other necessaries as may be required, pass the accounts and authorize their payment, and generally manage the affairs and administer the funds of the Association.

XVII. At a meeting prior to the Annual General Meeting the Council shall nominate a President, a Treasurer, two Secretaries, two Editors, and twelve Councillors, four of whom may not have served during the previous year, for recommendation to the Association for election in the ensuing year. Any member of the Council vacating office shall not be eligible for re-appointment as an ordinary member of Council until after the lapse of twelve months. The list as drawn up by the Council shall be sent to all members resident in Great Britain and Ireland at least four weeks before the date of the Annual General Meeting. It shall be competent for any member, on receipt of the recommendations of the Council, to add the name of a member or members of the Association to the list of candidates for election to the Council; such additional nominations, duly seconded, must be in the hands of one of the Secretaries not less than fourteen days before the Annual General Meeting.

The Secretary shall when necessary, and not less than seven days before the Annual General Meeting, issue to every member of the Association resident in Great Britain and Ireland a completed list of the proposed Officers and Council for

the year, indicating the names of the proposers and seconders of candidates other than the Council's nominees.

The election of new Officers and Council shall be conducted in the following manner:—At the Annual General Meeting each member present shall receive the list of Officers and Council proposed for the year. If no additional nominations have been received after the Council's recommendations, the list shall be put to the meeting and voted on by a show of hands and the result declared by the Chairman. If additional nominations have been received a ballot shall be taken; each member voting shall hand in person to one of the Secretaries a copy of the list on which has been indicated the names of those candidates whom the member voting desires to serve on the Council. When the ballot has been declared closed the Chairman shall appoint, from among the members present, two persons, not candidates for election, to serve as Scrutineers. In examining the lists handed in the Scrutineers shall set aside and take no account of any ballot paper which supports candidates in excess of the number provided for in Law IX, nor of any ballot paper which indicates the identity of the member voting. The Scrutineers shall report to the Chairman of the meeting the result of their scrutiny, and the Chairman, before the close of the meeting, shall announce the result of the ballot. In the case of an equality of votes for any candidates, the power of selection between them shall rest with the Chairman of the meeting and shall be exercised before announcing the result of the ballot.

XVIII. The Association shall meet at times and places to be decided by the Council.

At all Ordinary General Meetings ten shall form a quorum (see also Law XIX). All meetings shall be announced by circular addressed to each member resident in Great Britain and Ireland. At all Ordinary General Meetings the order of business shall be decided by the Chairman.

An Annual General Meeting shall, unless otherwise decided by the Council, be held on the date of the Ordinary General Meeting falling nearest to the beginning of the year.

At this Annual General Meeting the order of business shall be:

1. The reading of the minutes of the previous meeting.
2. The reading of a report of the Council on the work of the past year.
3. The statement of the Treasurer.
4. The election of Members.
5. The election of Officers and other Members of the Council.
6. Other business.

A Special General Meeting may be called to discuss or take action upon any matter affecting the interests of the Association.

A Special General Meeting shall be called either by the decision of the Council or at the request of at least ten members addressed to the Secretaries.

XIX. No new law shall be passed nor any standing law altered or added to, nor any other change in the constitution of the Association made except by a Special General Meeting of which for this purpose a fourteen days' notice must be sent to all Members resident in Great Britain and Ireland.

The requisition for such a Special General Meeting duly signed and stating in writing the laws proposed or the alteration desired, must be delivered to one of the

Secretaries, who shall within a reasonable period call such a meeting. The proposed new laws or alterations in the laws shall be printed in the circular convening the meeting.

At a Special General Meeting convened for the purpose of altering the constitution or amending the laws, fifteen shall form a quorum and no motion can be passed except by a two-thirds majority of those present and voting.

Note.

The following resolutions have been passed by the Council of the Association:

(1) That manuscripts reporting investigations dealing primarily with proprietary substances of which the composition or nature is not specified in such a way that the investigations can be repeated by other workers be not eligible for publication in the *Annals of Applied Biology*.

(2) That the mention of proprietary substances without specification of nature or composition be permissible in a manuscript provided that the particular substances are well known or standard and are only incidental in the work or contributory or subsidiary to the main theme or are used for purposes of control or comparative experiment.

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